CHOLINERGIC REGULATION OF RESTING CORONARY BLOOD FLOW IN THE DOMESTIC SWINE

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COWAN

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ABSTRACT

Title of Dissertation: Cholinergic Regulation of Resting Coronary Blood Flow in the Domestic Swine

Conrad L. Cowan, Doctor of Philosophy, 1989

Dissertation Directed by: Jack E. McKenzie, Ph.D., Associate Professor; Department of Physiology

Neural factors have been proposed to mediate tonic contraction of coronary arteries and thereby limit coronary blood flow. Recently, acetylcholine was shown to mediate vasoconstriction of isolated vessels from several species, including man. The aims of the present studies in domestic swine were to demonstrate cholinergic vasoconstriction *in vivo* and to determine whether resting cholinergic activity mediates basal coronary tone. In either chronically instrumented or acute preparations, coronary blood flow, cardiac function and blood gases were measured during either acetylcholine injection, muscarinic receptor blockade, or vagal ligation. Intracoronary injections of acetylcholine (0.5 - 3.0 μg) caused significant dose-dependent reductions (19-75%) in coronary flow and increases in coronary resistance. Atropine (200 μg) completely blocked these responses. Cholinergic mediation of basal coronary tone was initially evaluating by determined the effects of muscarinic blockade with intracoronary injection of atropine. Intracoronary atropine significantly increased coronary flow and decreased resistance in closed-chest, sedated, non-paced pigs. However, significant increases in myocardial oxygen consumption, due to

significant increases in heart rate and pressure rate product, masked potential flow increases due to removal of cholinergic tone. Therefore, an open-chest, anesthetized model was employed which afforded electrical pacing of the heart rate as a means to maintain myocardial oxygen consumption constant. In this model intracoronary injection of atropine had no significant effects on flow, resistance or other hemodynamic parameters with the exception of dP/dt. Finally, to insure that parasympathetically released acetylcholine was not overcoming the muscarinic blockade, vagal ligation was performed in an open-chest anesthetized model with the heart rate paced. Vagal ligation had no effect on coronary flow, coronary vascular resistance, or any other hemodynamic parameter. The power of these studies to detect significant change was in all cases greater than 70%. The present studies demonstrate acetylcholine induces a muscarinic vasoconstriction of coronary arteries in the domestic swine. However, these studies do not support a role for parasympathetic mediation of basal coronary vascular tone.

CHOLINERGIC REGULATION OF RESTING CORONARY BLOOD FLOW IN THE DOMESTIC SWINE

by

Conrad L. Cowan

Dissertation submitted to the Faculty of the Department of Physiology
Graduate Program of the Uniformed Services University
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SIGNIFICANCE

Regulation of coronary blood flow involves the dynamic interaction of many interrelated factors, including metabolic and neural chemical mediators as well as physical determinants such as perfusion pressure. Metabolic factors such as adenosine play an important role in matching coronary blood flow to myocardial oxygen demand [22,65,79]. When coronary flow falls below the metabolic needs of the myocardium, release of metabolic vasodilators increases. The metabolic vasodilators increase flow until a new balance between flow and demand is achieved. Also, both sympathetic and parasympathetic nerves exist on the coronary arteries and act to modulate flow [14,22,66]. Norepinephrine released from sympathetic nerve endings may cause either vasodilation or vasoconstriction depending on the predominance of either beta- or alpha-adrenoceptors, respectively [4,70]. Acetylcholine, the parasympathetic neurotransmitter, acting through muscarinic receptors has been thought to exclusively mediate vasodilation [22,92]. However, recent *in vitro* studies, of species other than the dog, have shown acetylcholine to be a potent vasoconstrictor [52,53,74,75]. Further evidence for cholinergic vasoconstriction is provided by clinical studies suggesting that parasympathetic activity may play a role in precipitating vasospasm associated with a subgroup of angina patients [17,44,78,104].

Metabolic factors such as adenosine mediate vasodilation to increase coronary blood flow. This ability implies a previous state of vasoconstriction of the vessels. Basal tone is described by Ginsburg [31]

in his studies of isolated human epicardial coronary arteries as "... spontaneous contractile tension generated by the vascular smooth muscle of a resting epicardial coronary artery, whether *in vitro* or *in vivo*. Although all vascular smooth muscle contraction is ultimately dependent on calcium, tone of the muscle is regulated and controlled by multiple physiological signals including nerves, circulating vasoactive hormones, and locally produced metabolites. Tone, together with the regulatory mechanisms controlling it, is quite different from that of skeletal or cardiac muscle. These muscles function in primarily on/off modes rather than states of tonic contraction as with vascular smooth muscle."[31] Many studies have tried to determine what mediates this resting tone on the coronary arteries. Adrenergic mechanisms have been the focus of previous studies of neural factors because alpha adrenergic vasoconstriction has been well documented [22,37,43,69]. However, the recent appreciation of acetylcholine as a vasoconstrictor, in species other than the dog, suggests that tonic cholinergic activity could contribute to resting coronary tone.

PHYSICAL REGULATION OF CORONARY FLOW

Fluid flow through a rigid tube was described mathematically by Poiseuille to be equal to $(\pi(P1\text{-}P2)r^4)/8\eta l$, where P1-P2 is the pressure gradient along the tube, r is the radius of the tube, η is the viscosity of the fluid, and l is the length of the tube. Application of Poiseuille's law to the cardiovascular system, however, can only be done qualitatively because assumptions of the law are violated. Such

violations include; blood vessels are not rigid, they branch, the blood is a non-Newtonian fluid, and flow is not always laminar, nor is it steady [6]. However, the general relationship of Poiseuille's law, flow being equal to the pressure gradient (P1-P2) divided by resistance ($R \approx 8\eta l/\pi r^4$), is very helpful in understanding the blood flow through the cardiovascular system.

In the coronary circulation the pressure gradient during diastole is the difference between arterial pressure and coronary sinus pressure. However, this pressure gradient varies during the cardiac cycle due to the unique nature of the coronary vessels which have the additional factor of the extravascular compression of the contracting myocardium. Thus, during systole, the contraction of the myocardium compresses the coronary vessels, beginning first at the lowest pressure veins and moving toward the higher pressure arteries. In this sense, the coronary arteries have been proposed to behave as Starling resistors. This changes the pressure gradient to the difference between arterial pressure and intramyocardial tissue pressure. Since myocardial tissue pressure may equal or exceed the driving pressure of mean arterial pressure, coronary flow falls to zero during systole. Flow dependence on tissue pressure, rather than venous pressure, has been termed the "waterfall effect". Thus, the phasic compressive nature of the contracting myocardium produces a phasic coronary blood flow with the maximum flow occurring during diastole. The effect of these myocardial compressive forces remain relatively constant at a given intraventricular pressure and heart rate, and therefore play a relatively minor role in the direct instantaneous regulation of coronary

blood flow.

As Poiseuille's equation indicates, the flow of blood into the coronary vessels can be altered by changes in either the pressure gradient or vessel radius (assuming blood viscosity and vessel length remain constant). Since flow is proportional to the fourth power of the radius of the vessel, altering vascular diameter is the most efficient means of regulating coronary blood flow. Alterations in vessel radius and hence vascular resistance are produced by neural and metabolic vasoactive substances acting to regulate coronary blood flow. Vasoactive metabolites generally produce vasodilation while neural transmitters produce either vasodilation or vasoconstriction.

METABOLIC REGULATION OF CORONARY BLOOD FLOW

The release of metabolic vasoactive agents occurs when there is a reduction in the supply to demand ratio, as occurs during the increased myocardial metabolic state of exercise [65]. Metabolic vasoactive agents act to increase flow to maintain adequate oxygen delivery [79]. During increased metabolic states, coronary blood flow must increase to deliver more oxygen for two reasons. First, the cardiac muscle cells are primarily aerobic cells with very little ability to sustain anaerobic metabolism [79]. Therefore, to maintain their function, myocytes must receive oxygen at rates corresponding to the amount of oxygen they are consuming and therefore, work they are performing. Secondly, even under conditions of rest the heart extracts approximately 75% of the oxygen delivered by the blood, leaving little oxygen reserve available through

increased extraction [21]. Therefore, to meet increased oxygen requirements, oxygen delivery must be increased through increased flow [79]. Vasoactive metabolites are released from the myocardium when oxygen supply falls below myocardial oxygen demand [3,85]. Without adequate oxygen to sustain aerobic metabolism myocytes are unable to convert low energy metabolic endproducts back to their high energy states. As the amounts of metabolic end-products within a cell rises they are released into the interstitium [3]. The concentrations of these metabolites increase in the interstitium and cause relaxation of the vascular smooth muscle. This relaxation allows increased vessel radius and thereby allows increased blood flow and hence oxygen delivery. Oxygen delivery and metabolite concentration in the interstitium will then reach a new equilibrium to meet the increased metabolic demand.

NEURAL REGULATION OF CORONARY BLOOD FLOW

Anatomy

Metabolic regulation of coronary blood flow is influenced by neurotransmitters released by the autonomic nervous system [69]. Neural innervation of both sympathetic and parasympathetic origin has been demonstrated, histologically and physiologically, on the coronary arteries of many mammals including, dogs, pigs, monkeys, and man [14,52,80].

(Gross)

McKibben and Getty [66] have described in the pig the path of the cardiac nerves arising from the sympathetic ganglia and the vagus

nerves. Those nerves arising from the left side of the spinal cord pass primarily to the caudal right surface of the heart while those of the right proceed to the cranial right and left sides. Innervation from one side was observed to be augmented by the opposite side in the cardiac plexus for both sympathetic and parasympathetic nerves. A portion of the right caudal vagal cardiac nerve passes through the cardiac plexus to the left coronary groove and longitudinal sulcus, supplying cranial and ventral portions of the left auricle as well as the interventricular septum from the left side.

(Microscopic)

Histochemical examination of the dog heart confirms the adrenergic and cholinergic innervation of the coronary vessels of the left myocardium [14,80]. Adrenergic cardiac nerves were found to terminate in a perivascular plexus in the adventitial layer of the coronary arteries. Acetylcholinesterase staining was used as an indication of muscarinic receptors and the possible existence of cholinergic nerves. Such staining was found along the medial-adventitial border in left anterior descending and circumflex coronary arteries [14,80]. The nerves formed a perivascular network which was not limited to the adventitial layers. Although nerves were not found to penetrate into the media, stimulation of the smooth muscle cells in the outer layers of the vessels could be communicated to the more luminal cells through an intracellular network of gap-junctions such as that described by Ginsburg [30] in human coronary vascular smooth muscle.

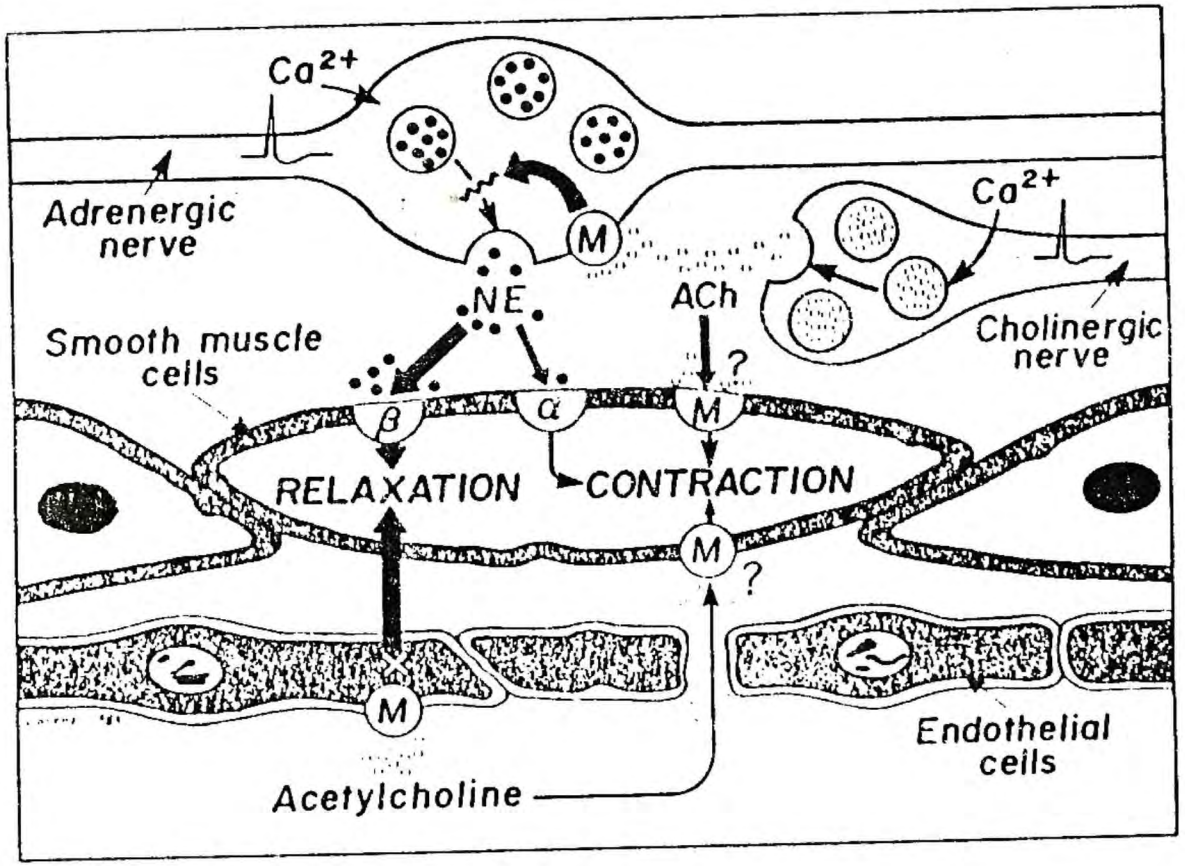
Vagal and sympathetic ligation, performed three weeks prior to the histological staining, removed adrenergic innervation but left

cholinergic innervation intact [14]. This is consistent with the classical concept of sympathetic postganglionic neurons being rather long and diffuse whereas parasympathetic neurons are relatively short and discrete. Thus sympathetic postganglionic neurons may originate outside the heart and therefore be subject to ligation. Conversely, parasympathetic postganglionic neurons originate very close to the effector organ, the coronary vessels in this case, and would not be damaged by vagal ligation.

Receptor Subtypes

Neural regulation of blood flow is mediated by neurotransmitters which act on receptors of the vascular smooth muscle to induce relaxation or contraction. Figure 1 depicts adrenergic and cholinergic innervation of coronary vascular smooth muscle. Adrenergic receptors have been characterized into two subgroups, alpha and beta, due to their different responses to various catecholamines [102]. Each group consists of at least two subgroups ($alpha_{1,2}$ and $beta_{1,2}$, respectively) again based on relative selectivity to agonists and antagonists [102]. Coronary vasoconstriction has been shown to be mediated postsynaptically by both $alpha_1$ and $alpha_2$ receptor subtypes [70]. $Alpha_2$ receptors are also found presynaptically on adrenergic nerve terminals and act to inhibit the release of norepinephrine [70]. Conflicting reports have clouded the characterization of the vascular beta adrenergic subgroup which mediates vasodilation. *In vivo* studies [39] support $beta_2$ mediated vasodilation while *in vitro* [10,70] studies characterize the receptor as $beta_1$. Adrenergic receptors on the myocytes are $beta_1$ [72].

Figure 1. Presynaptic, vascular smooth muscle and endothelial sites of action for norepinephrine and acetylcholine released from their respective adrenergic and cholinergic neurons.(Taken from Vanhoutte and Cohen [96])



Parasympathetic cholinergic receptors have also been characterized into two groups, muscarinic and nicotinic, based on their responsiveness to various agonists [91]. Nicotinic receptors are found systemically primarily at preganglionic nerve terminals and at motor-end plates of skeletal muscle. Muscarinic receptors have been characterized into M_1 and M_2 subgroups based on the affinity of the agonists, McN-A-343 and bethanecol. Atropine readily antagonizes muscarinic stimulation by either agonist. M_1 receptors are found primarily on adrenergic and cholinergic neurones and are preferentially stimulated by McN-A-343. Their stimulation inhibits the release of neurotransmitter [11,58]. Muscarinic receptors on the smooth muscle are M_2 and are preferentially stimulated by bethanecol [101]. Stimulation of muscarinic receptors of porcine coronary smooth muscle does not alter membrane potential or resistance but does mobilize intracellular calcium [50].

It is through these receptors that the sympathetic neurotransmitter norepinephrine and the parasympathetic neurotransmitter acetylcholine, mediate their effects. Thus, there is ample anatomic evidence linking parasympathetic innervation and cholinergic receptors with the coronary arteries to support a role for their control of coronary blood flow.

Adrenergic Effects on Coronary Blood Flow

Determination of the direct effects of neural activity on coronary tone and hence coronary blood flow were initially difficult to determine due to the concomitant changes in myocardial performance. Early studies on adrenergic control of coronary blood flow have been extensively

reviewed by Ross [84] and Feigl [21]. Early investigations of sympathetic effects, which employed intravenous infusion of norepinephrine or stellate ganglion stimulation, resulted in vasodilation [35]. However, these manipulations also caused increased myocardial work and oxygen consumption. Therefore, the reported increased coronary blood flow could also be explained by a metabolically mediated vasodilation. As flow measurement techniques improved, investigators reported a biphasic response to sympathetic stimulation. Following catecholamine infusion or stellate ganglion stimulation, blood flow initially decreased and then dramatically increased. These investigators concluded that the initial direct action of adrenergic stimulation was vasoconstriction which was overcome by metabolic vasodilation to yield an overall increase in coronary blood flow [5].

Coronary blood flow is regulated via competition between metabolic and neural factors. Several studies have investigated the ability of sympathetic activity to limit flow during the increased metabolic demand which occurs during exercise [38,42,43,73]. In these cases coronary flow increased approximately 30% following adrenergic blockade. The findings from these studies indicate adrenergic flow restriction during increased myocardial demand imposed by exercise. Feigl demonstrated that the sympathetic limitation of flow also results in a reduced oxygen delivery thereby causing a reduction in the myocardial supply to demand ratio [20]. Following adrenergic blockade, coronary sinus oxygen content rose and myocardial oxygen extraction decreased. The results of these studies suggest that adrenergic vasoconstriction successfully competes with metabolic vasodilation, under conditions of high sympathetic activity,

to reduce the supply of oxygen below the level determined by metabolic regulation.

The involvement of adrenergic vasoconstriction in mediating basal coronary tone, however, has been quite controversial [37,43,51,73]. Administration of phentolamine, a mixed alpha adrenergic antagonist, blocks the sympathetic vasoconstriction which limits active hyperemia in the above studies, but does not cause an increase in basal coronary blood flow. Macho [63] compared the response of two $alpha_1$ specific blockers, prazosin and trimazosin, with phentolamine. Neither phentolamine, nor prazosin produced significant changes in coronary blood flow. Trimazosin caused a slight yet significant increase in flow. However, coronary sinus oxygen content and myocardial oxygen consumption were not reported, leaving doubt as to whether the change in flow was metabolically mediated. Schwartz and Stone [88] observed an increased reactive hyperemia following blockade of sympathetic input through section of the left stellate ganglia. Similar results were obtained following $alpha_{1,2}$ blockade with phentolamine. They concluded that tonic sympathetic activity limited the ability of the coronary arteries to vasodilate. Holtz et al. [46] selectively sympathectomized a portion of myocardium using 6-hydroxydopamine. Basal coronary blood flow in the sympathectomized region was significantly greater than flow in the innervated region. Chillian et al. [9] and Griggs et al.[36] however, found no evidence for sympathetic tone using a similar, yet less damaging technique than Holtz et al. [46], which used phenol rather than 6-hydroxydopamine. Although adrenergic stimulation has been shown to limit coronary flow during increased myocardial performance thereby

also acting to reduce the supply to-demand-ratio, evidence indicating a role in regulating basal coronary tone is not conclusive [97].

Studies of adrenergic influence on coronary blood flow provide support for neural regulation of coronary blood flow. They also support the concept that neural activity of a vasoconstrictor nature may mediate basal coronary tone, although they are unable to provide conclusive evidence that it is provided by sympathetic innervation.

Cholinergic Effects on Coronary Blood Flow

Difficulty similar to that of the adrenergic studies is experienced in determining parasympathetic influence *in vivo*. Vagal stimulation leads to a reduction in coronary blood flow [35]. A concomitant decrease in heart rate and thus myocardial function which could account for the flow changes. Vagal stimulation studies by Feigl [22] and by Tiedt and Religa [92] resulted in increased coronary blood flow when heart rate and therefore myocardial metabolism were held constant with pacing. The increased flow was concluded to be the result of direct cholinergic vasodilation. The vasodilatory response to vagal stimulation was blocked by atropine, indicating the response was mediated through muscarinic receptors.

In vitro studies on helical strips of rabbit aorta, initially conflicted with the early *in vivo* studies, finding only contraction upon addition of acetylcholine to the perfusate [24,25]. These conflicting findings were resolved by Furchgott and Zawadzki [26] who were able to demonstrate acetylcholine mediated relaxation of rabbit aorta ring.

They deduced that stimulation of the vascular endothelial muscarinic receptors initiates production and release of an endothelial vasodilating agent [26]. They concluded that the earlier *in vitro* studies had unknowingly damaged the vascular endothelium while preparing the tissue. Endothelium mediated coronary vasodilation has been confirmed in coronary artery ring segments from dogs [52]. These and other supporting studies, primarily from dogs, formed the widely accepted view that acetylcholine and vagal stimulation produce an endothelium dependent vasodilation [103].

Although the dog has been used as a model for the human condition many times in the past, extrapolation of this view of acetylcholine as a coronary vasodilator to humans has been misleading. Some clinical studies have based their conclusions on this view without consideration of possible species differences [8,18,47,106,107]. However, a 1950 study by Smith [89] in human coronary arteries found only vasoconstriction in response to acetylcholine. Recent studies in other species, including man, have found that an endothelium dependent vasodilation is not the response of coronary arteries to acetylcholine in many species.

Exogenous acetylcholine [30,32,33,50,52,74,75] has been shown to be a potent vasoconstrictor of porcine coronary arteries *in vitro*. Both epicardial conductance [33,74] and resistance vessels [75] demonstrated dose dependent vasoconstriction to acetylcholine. Relaxation induced by the endothelium dependent vasodilator, substance P, indicated that damage of the endothelium was not responsible for the lack of relaxation in response to acetylcholine [33,75]. Also, removal of the endothelium had no effect on this response [33]. This vasoconstriction was antagonized by

atropine indicating that it was mediated by muscarinic receptors.

Cohen et al. [11] have shown that acetylcholine presynaptically inhibits release of norepinephrine from adrenergic nerves. They suggest that this may inhibit beta-adrenergically mediated vasodilation. However, several studies have shown that acetylcholine mediated vasoconstriction is unaffected by adrenergic blockade [27,54,86,87]. Thus, it is unlikely that such a mechanism plays a role in the vasoconstriction induced by acetylcholine in porcine coronary arteries.

Kalsner [52] and Ginsburg [32] have confirmed the species specific response to acetylcholine in studies which compared the responses of porcine, canine, and bovine to those of human coronary arteries under similar conditions. Dose dependent constriction to acetylcholine was observed in all the isolated vessels from all species except canine which responded with vasodilation. The response to acetylcholine was unchanged after the removal of the endothelium, except in the canine coronaries which then either had no response or weak constriction. The lack of endothelial involvement implies that acetylcholine's vasoconstrictor actions are mediated through muscarinic receptors on the vascular smooth muscle. These comparative studies demonstrate that an endothelium independent vasoconstriction appears to be the predominant response to acetylcholine in many species including humans.

Ludmer et al.[62] have suggested that atherosclerosis may reverse cholinergic vasodilation to vasoconstriction. Intracoronary injection of acetylcholine into patients with normal coronary arteries produced vasodilation. However, when given to patients with atherosclerosis, acetylcholine produced marked (>66%) vasoconstriction. Although the

coronary arteries in Ginsburg's [32] study were obtained from cardiac transplant patients, those of Kalsner's [52] study were obtained from patients who died from non-cardiac disorders and therefore the vasoconstriction was not likely due to atherosclerosis. Fostermann et al. [23] observed vasoconstriction to acetylcholine in human coronary arteries free of atherosclerosis. Removal of the endothelium abolished the relaxation to substance P and A23187 but not the vasoconstriction to acetylcholine. In contrast to Ludmer's findings, several clinical studies have demonstrated coronary vasoconstriction to acetylcholine in patients with angiographically normal coronary arteries [68,77,78,104]. Although atherosclerosis may alter the sensitivity of coronary vessels to acetylcholine it does not appear to be required for vasoconstriction.

Several clinical studies have demonstrated coronary vasoconstriction to acetylcholine in patients with angiographically normal coronary arteries [68,77,78,104]. This has led to the suggestion that parasympathetic activity may play a role in the vasospasm associated with the clinical syndrome of Prinzmetal's angina [81]. These patients experience the chest pain of angina when parasympathetic activity is highest; at rest, and often in the early morning hours when the patient is asleep. Bradyarrhythmias and various degrees of atrioventricular block also are characteristic of these attacks, again suggesting a high degree of parasympathetic activity [1,44]. Studies have found that such anginal attacks can be induced by injections of methacholine or acetylcholine [47,55,68,78,106]. These characteristics of Prinzmetal's angina suggest a high degree of parasympathetic activity indicating a role for acetylcholine in mediating the extreme coronary vasoconstriction, termed

vasospasm, which has been shown to be cause of the reduction in coronary flow [44,81,82].

Endogenous acetylcholine, released from parasympathetic nerves, vasoconstricts coronary arteries *in vitro* [27,54,108]. Kalsner [54] and Furusho et al. [27] report similar findings during perivascular nerve stimulation of bovine and porcine perfused isolated epicardial arteries, respectively. Frequency dependent vasoconstriction was induced with stimulation from 5-20 Hz with 50V. Much like exogenously applied acetylcholine these vasoconstrictions were not affected by phentolamine or propranolol but were greatly attenuated by atropine. Neostigmine [27] and physostigmine [54], cholinesterase inhibitors, augmented the vasoconstrictor response. Acetylcholine also induced dose dependent vasoconstriction in both studies whereas norepinephrine either produced vasodilation (bovine) or had no effect (porcine). Similar findings of vasoconstriction with electrical stimulation have been reported in strips of human coronary artery [53]. These studies suggest that parasympathetic activity would mediate vasoconstriction *in vivo*.

In vivo studies provide support for these findings in isolated vessels. Intracoronary nicotine produced coronary vasoconstriction in conscious calves [108]. The mechanism of this response, as suggested by the authors, is nicotinic stimulation of acetylcholine release from postganglionic parasympathetic nerves located in the medial-adventitial layer of the coronary arteries. This vasoconstriction was unaffected by either alpha, beta, or combined adrenergic blockade but was blocked by atropine or hexamethonium. Since adrenergic blockade had no effect on the response to acetylcholine it is unlikely that acetylcholine's

effects are produced through presynaptic alteration of sympathetic output as suggested by Cohen [11]. This implies a neurally innervated muscarinic response which is not affected by, or mediated through, alteration of adrenergic responses. Although no coronary bed has exhibited all the characteristics of the human coronary circulation [30,32] the similarity of porcine innervation and vascularization as well their shared endothelium independent vasoconstriction to acetylcholine makes pigs a good model for the study of parasympathetic influence [49].

Cholinergic vasoconstriction *in vitro* has become a well documented phenomena much as is adrenergic vasoconstriction. The ability of this vasoconstriction to limit coronary blood flow when stimulated also parallels the similar adrenergic finding. These findings coupled with the high parasympathetic activity at rest which acts to maintain a lower heart rate [12,64] suggest that cholinergic influences might contribute to basal coronary tone.

RATIONALE

Parasympathetic activity is known to play a role in regulating coronary blood flow. However, recent studies have found evidence which suggests that the accepted view of acetylcholine as a coronary vasodilator may not apply to all species. For example, Kalsner [52] and Ginsburg [32] demonstrated that acetylcholine produced an endothelium dependent vasodilation in canine coronary arteries while producing only vasoconstriction in isolated vessels from various other species, including man. The purpose of these studies was to determine whether tonic cholinergic activity to the coronary arteries results in tonic vasoconstriction, acting to limit resting coronary blood flow.

The porcine model was chosen for it's similarity to the human condition. In particular, porcine coronary arteries are similar to human coronary arteries with respect to their response to various agonists, including acetylcholine. Also, the neural innervation of the porcine coronary arteries and the lack of extensive coronary artery collateralization is similar to that of human coronary arteries. These similarities make results from the porcine model better for extrapolation to the human condition as compared to those from the canine model. Therefore, swine were used in the present studies to investigate the role of cholinergic activity in regulating resting coronary blood flow.

Determination of resting coronary blood flow limitation by cholinergic activity, initially required *in vivo* demonstration of the

vasoconstrictor nature of acetylcholine. Experiments designed to test the effects of acetylcholine on coronary blood flow involved measurement of coronary blood flow during injection of acetylcholine into the left anterior descending coronary artery.

The effects of tonic parasympathetic activity on resting coronary blood flow were investigated by evaluating the effects of removal of parasympathetic influence. Parasympathetic influence on the coronary arteries was removed in two studies by muscarinic blockade with the intracoronary administration of atropine, and in a third study by vagal ligation. Removal of tonic cholinergic vasoconstriction would be expected to result in increased coronary blood flow and decreased arterial-coronary sinus oxygen content difference.

The results of this research indicate that although acetylcholine is a potent vasoconstrictor, tonic parasympathetic activity does not limit resting coronary blood flow under normal conditions in the swine. This finding adds new understanding of the role of the parasympathetic nervous system in regulating coronary blood flow *in vivo*. The similarities that exist between the coronary arteries of swine and man suggests that the present findings may also extend to human coronary physiology. This extension may aid in the understanding of the pathophysiology of Prinzmetal's angina, a syndrome of coronary vasospasm in associated with periods of high parasympathetic activity.

MATERIALS AND METHODS

SURGICAL PREPARATION

Healthy domestic swine of either sex, weighing 20-25 kg, were used in all studies. Their use and handling conformed to the guidelines established by the United States Department of Health and Human Services' Guide for the Care and Use of Laboratory Animals as well as the Uniformed Services University of the Health Sciences' Instruction 3202. Every effort was taken to insure that these animals were treated humanely.

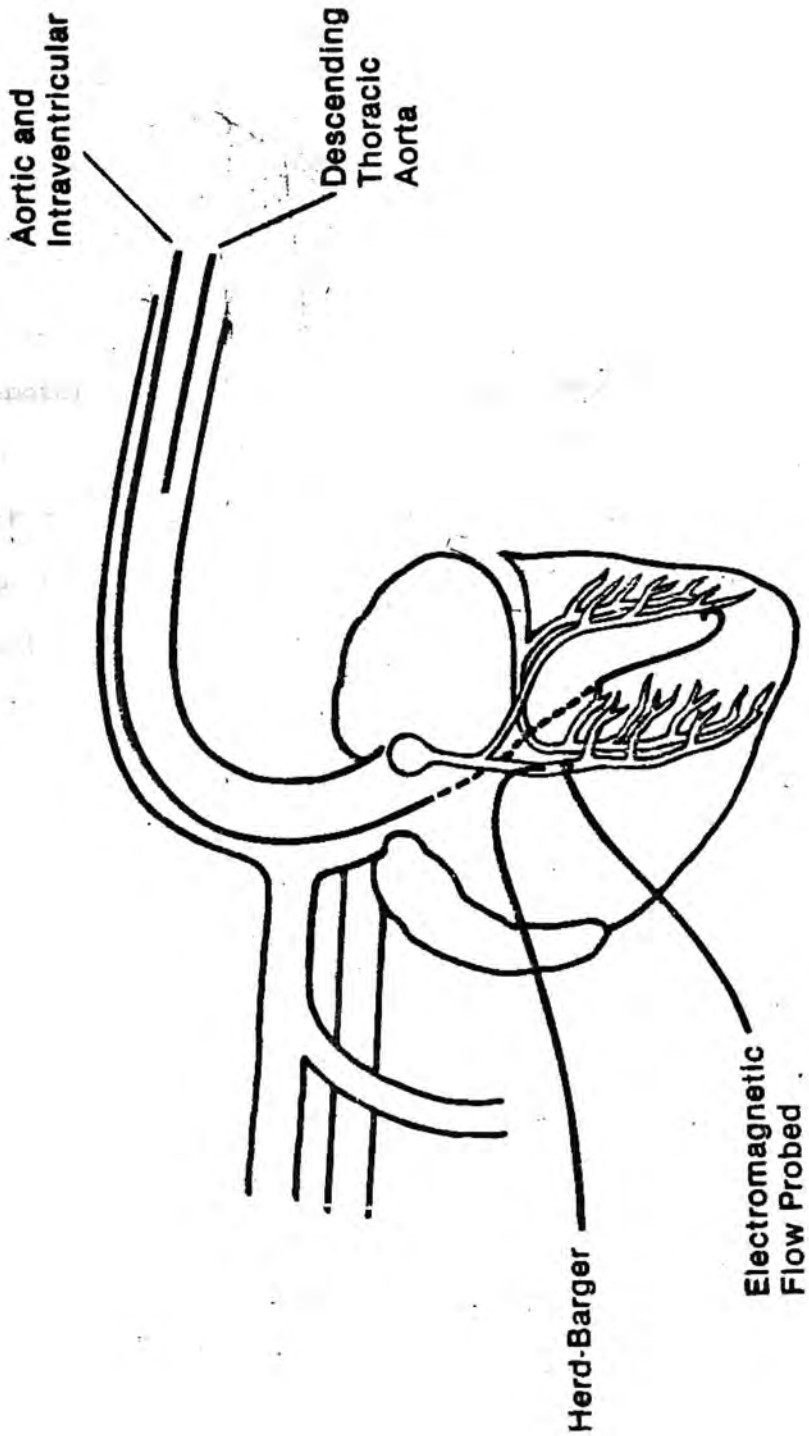
A basic surgical procedure was followed in several of the following studies. The details of this procedure are described in length for the first study and then referred to in following studies. Only exceptions or additional procedures will be described for subsequent studies.

Acetylcholine Dose-Response Studies;

The animals were initially sedated with a bolus dose of ketamine (600 mg, i.m.). An anesthetic plane was achieved and maintained by a solution of alpha-chloralose (80 mg/kg) and urethane (800 mg/kg)[34] administered intravenously through an angiocath (21 gauge, Deseret) in a marginal ear vein. Supplemental doses were administered as necessary.

The pigs were then intubated to maintain open airways and provide for positive pressure ventilation and oxygen supplementation during open chest procedures (Harvard Apparatus, model 613). Fluid filled catheters connected to Statham pressure transducers (model P23Gb, connected to Hewlett-Packard 14060D transducer adapter) were inserted into both right and left femoral arteries. Before and periodically during the experiment the transducers were balanced to atmospheric pressure and the gain calibrated against a mercury filled manometer. A 7F intraventricular catheter (USCI 006112) was introduced into the left femoral artery and advanced into the ventricle as determined by the characteristic pressure recording (Figure 2). The right femoral catheter (PE-160, Clay Adams) was advanced into the descending thoracic aorta for measurement of mean and phasic arterial pressure and arterial blood withdrawal. A left lateral thoracotomy at the fourth intercostal space exposed the heart. The pericardium was cut and sewn to the chest wall to form a cradle. The left anterior descending coronary artery was carefully isolated and catheterized with a modified Herd-Barger catheter [41]. The technique for chronic catheterization of Herd and Barger was modified with the use of tygon tubing (Fisher Scientific, 0.04 in. I.D., 0.07 in. O.D.) pulled to a very thin diameter (<1mm) after warming in oil. This extremely fine tubing tied to 5-0 Prolene suture (Ethicon) was then inserted and pulled into the vessel, allowing for selective intracoronary (i.c.) injection of drugs. A precalibrated circumferential electromagnetic flow probe (Carolina Medical Electronics, model 501 Electromagnetic Blood Flowmeter, King, N.C.) was then placed distal to the coronary catheter for measurement of coronary

Figure 2. Cardiac catheter placement for the Acetylcholine Dose-Response Study.



blood flow.

Atropine Dose-Response Study

The surgical procedures for this study were similar to the Acetylcholine Dose-Response Study with the following exceptions. Only the right femoral artery was exposed through a small incision in the medial thigh. A catheter (Millar Instruments SPC-780C, Houston, TX), equipped with two micro-manometers positioned 1.5 and 6.5 centimeters from the tip, was advanced through the femoral artery and into the left ventricle such that one manometer was located in the ventricle and one in the aorta. Prior to the experiment the catheter was calibrated and internal calibration signals were verified using a specially designed sleeve which covers the manometers and allows calibration against a mercury filled manometer. Catheter position was verified by characteristic intraventricular and aortic pressure recordings. The rate of intraventricular pressure change (dP/dt), and heart rate were also determined from these pressure measurements.

Effect of Basal Muscarinic Blockade

Sterile Surgery for closed-chest studies;

Following initial sedation with ketamine (600mg, i.m.), anesthesia was achieved and maintained by inhalation of isoflurane (1.5% in oxygen). Under sterile conditions the chest was opened through a left lateral thoracotomy at the fourth intercostal space. A tygon catheter (Fisher Scientific, 0.04 in. I.D., 0.07 in. O.D.) was introduced into the left mammary artery and advanced to the axillary artery (Figure 3).

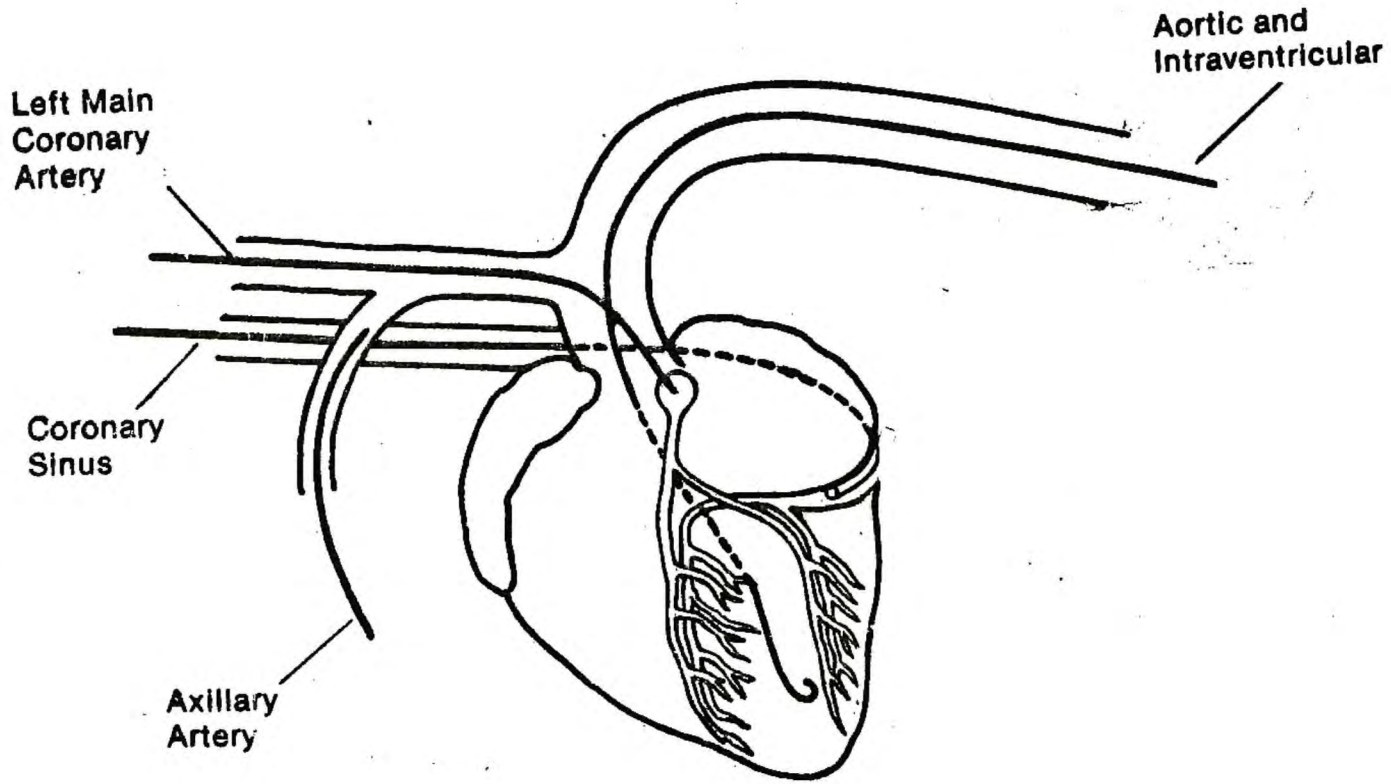
The hemiazygous vein was then cannulated with tygon tubing. The catheter tip was advanced 3-4 centimeters into the coronary sinus and position was visually verified. Another tygon catheter was introduced into a small branch of the pulmonary vein and secured with ligatures. These catheters allowed, arterial blood withdrawal, drug infusion or back-up coronary sinus blood withdrawal, and microsphere injection, respectively. The tubing was tunnelled subcutaneously from the fifth intercostal space and exteriorized at a point between the scapulae. A 24F chest tube (Argyle) was exteriorized posterior to the opening of the chest to aid in drainage and development of negative intrathoracic pressure. The chest was then closed and a negative intrathoracic pressure created by withdrawal through the chest tube.

Following the procedures in the chest, an incision was made in the neck for isolation of the carotid artery and jugular vein. Silastic precatheters (0.132 in. I.D., 0.183 in. O.D., Dow Corning) were then secured in these vessels and exteriorized through the incision which was then closed around the catheters. A volume of heparin (1000 units/ml), equal to the volume of the catheter as determined by withdrawal, was injected into all catheters to prevent clot formation within the catheters. The catheters were then secured by sterile gauze and self-adhesive bandages to the animals neck.

The animals were then allowed to recover and were used for collection of data four to seven days following surgery. Both prior to and following these surgical procedures the animal received an antibiotic regimen of Ampicillin (10mg/kg, i.m., twice daily).

On the day of the experiment, the pigs were sedated with an

Figure 3. Cardiac catheter placement for the Basal Effects of Muscarinic Blockade : Closed-Chest Study



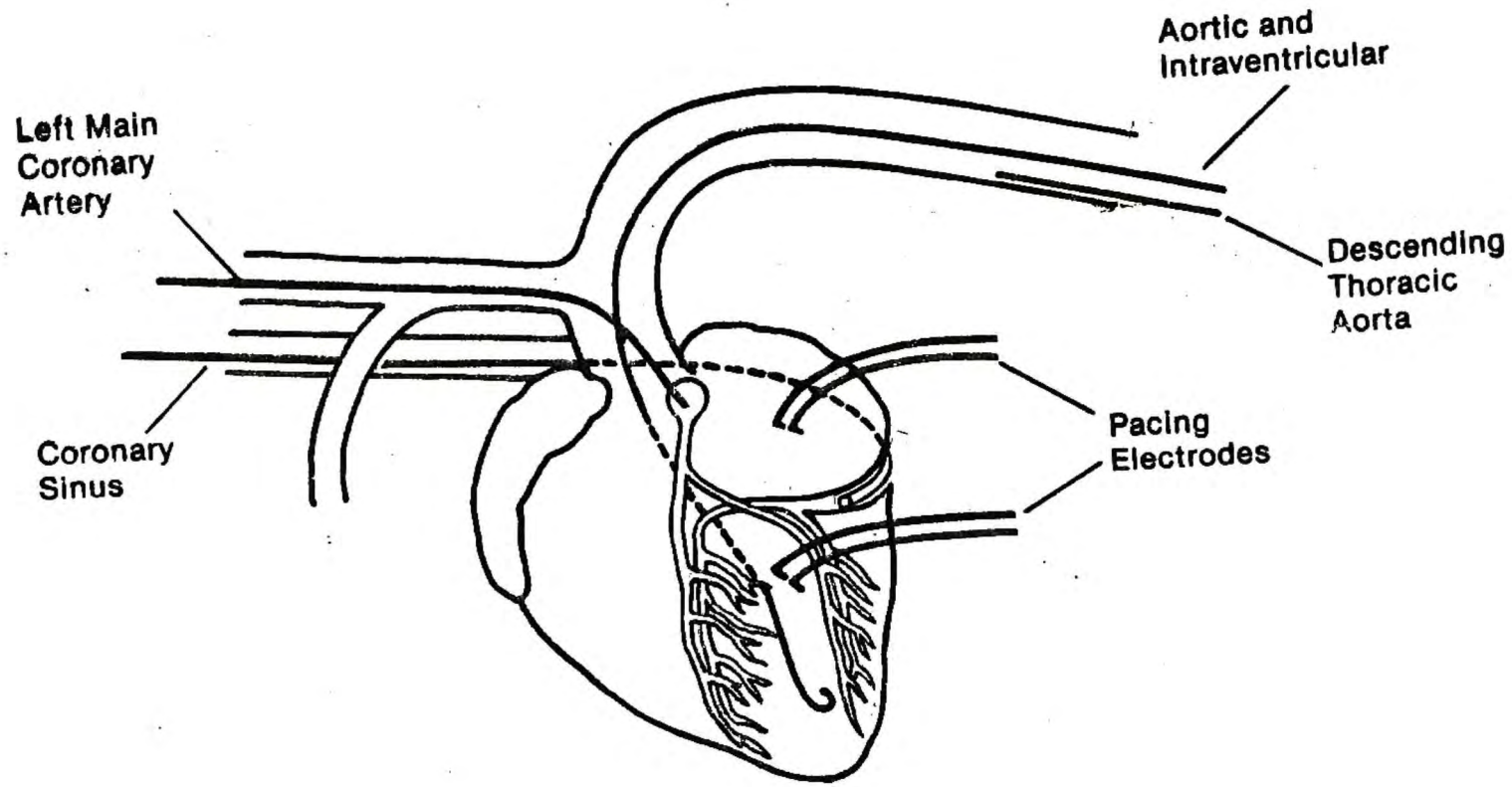
initial bolus dose of ketamine (600 mg i.m.) and sedation was maintained with diazepam (1-2 mg/kg/hr, i.v.). After withdrawing through the silastic precatheters to remove the heparin and any clots, a 5F multipurpose A-2 catheter (Cordis, 521-514) was introduced under fluoroscopy into the left main coronary artery through the carotid artery and a 7.5F Sones coronary sinus catheter (USCI 007561) into the coronary sinus through the jugular vein (Figure 3). Position of the coronary catheters was verified by injection of radio-opaque dye (Renographin^{tm},Squibb). These catheters provided for specific injections into the left main coronary artery and selective coronary sinus blood withdrawal, respectively. The right medial thigh was then locally anesthetized by subcutaneous injection of lidocaine. The femoral artery was isolated and catheterized as described previously (four animals received fluid filled catheters as in the Acetylcholine Dose-Response Study, two received the Millar as described in the Atropine Dose Response Study). Position of all catheters was verified upon autopsy. Postmortem the coronary sinus catheter tip was found to lie 3-4 cm beyond the coronary sinus ostium, sufficient distance to selectively sample coronary venous blood [55].

Effect of Basal Muscarinic Blockade

Pacing studies;

The surgical procedure followed for the Atropine Dose-Response Study was used in this study with the following exceptions. A left sagittal incision was made in the neck and the external jugular vein and the carotid artery were isolated, with care taken not to touch the vagal

Figure 4. Cardiac catheter placement for Basal Effects of Muscarinic Blockade : Pacing Study



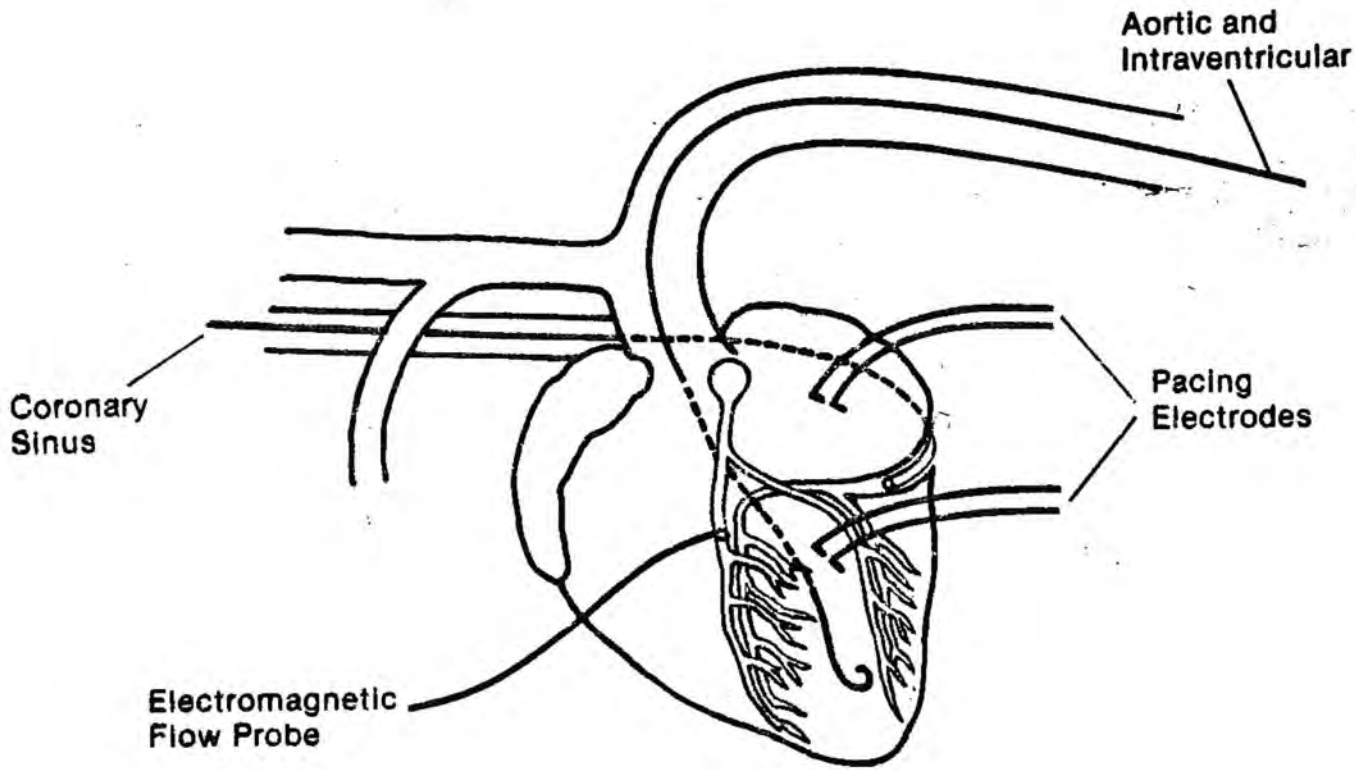
nerves. A 5F multipurpose A-2 (Cordis) catheter was introduced under fluoroscopy into the left main coronary artery through the carotid artery and a 7.5F Sones coronary sinus catheter into the coronary sinus through the jugular vein (Figure 4). Position of the coronary catheters was verified by injection of radio-opaque dye (Renographin^{tm},Squibb). Once again, these catheters provided specific intracoronary injections and coronary sinus blood withdrawal, respectively.

The heart was exposed as previously described for the Acetylcholine studies. The catheter to be used for microsphere injection was placed in the left atrial appendage rather than the pulmonary vein. As in the closed-chest study, specific intracoronary injections were made through a catheter in the left main coronary artery. To control heart rate without altering function, curved, pointed, platinum electrodes were placed in pairs on the left atria and ventricle for sequential atrial-ventricular pacing of the heart.

Vagal Ligation Studies;

Animals in this study were surgically instrumented as in the Atropine-Dose Response Studies with the addition of vagal isolation and coronary sinus catheter placement. This isolation involved a frontal midline incision of the neck, followed by a 4 centimeter transverse incision on either side from midline to the shoulder. This pattern allowed maximum exposure of the carotid sheath, minimizing trauma to the vagus nerves during their isolation. The nerves were isolated using custom made curved glass instruments to avoid depolarization or trauma. The nerves were isolated prior to the surgical procedures in the chest

Figure 5. Cardiac catheter placement for Vagal Ligation Study.



and were marked loosely with a suture. Until the beginning of the experiment, the nerves were kept moist with a suspension of mineral oil and saline and packed with similarly moistened gauze. A 7.5F Sones catheter was guided into the coronary sinus under fluoroscopy as described in the Basal Muscarinic Blockade studies (Figure 5). This catheter allowed sampling of coronary venous blood.

EXPERIMENTAL DESIGN

Following the surgical procedures, arterial blood samples were withdrawn, before each experiment, into ice-cold heparinized glass syringes for determination of pH, pO2, pCO2 (ABL3, Radiometer/Copenhagen, Cleveland, Ohio), oxygen content (OSM3, Radiometer/Copenhagen, Cleveland, Ohio) and hematocrit. If necessary, blood gases were corrected to within normal limits by adjustment of ventilatory rate or supplementation of oxygen. The animals were then allowed to stabilize for at least 30 minutes. Animals rectal temperature was monitored (Yellow Springs, 411A) and maintained between 37-38°C by a circulating water heating pad (American Hamilton, K-20). Unless otherwise noted, the hemodynamic parameters of arterial and intraventricular pressure, the first derivative of intraventricular pressure (dP/dt), and heart rate, as well as Lead II of the electrocardiogram, were continuously recorded on a Hewlett Packard 8-channel chart recorder (7758A). Pressure-rate product, myocardial oxygen consumption and extraction, were calculated from measured parameters.

Acetylcholine Study (n=5)

Following recording of control conditions, bolus doses of acetylcholine (in a range of 0.25 -3.0 μg) were injected in a random fashion into the left anterior descending coronary artery via the modified Herd-Barger catheter. Each injection was followed by a 0.5 ml pH-balanced saline flush. Alternately, volumes of saline equivalent to the total injection for each dose (0.25-1.0ml) were injected for standardization of the effect of the flush and drug. Following all doses of acetylcholine, a maximal muscarinic blocking dose of atropine (200 μg) was injected intracoronary to determine whether the effects of acetylcholine were mediated by muscarinic receptors. Injections of acetylcholine (4.0 -30.0 μg) were given following atropine to evaluate the competitive nature of the blockade. Responses in all parameters to acetylcholine were determined during the maximum change following injection.

Atropine Dose-Response Study (n=3)

To determine a dose of atropine which could block high doses of exogenous acetylcholine without causing systemic hemodynamic effects, an atropine dose-response curve was performed. Atropine was administered into the left anterior descending coronary artery. Flow was measured in this artery by the electromagnetic flow probe. To provide increasing log molar concentrations of atropine ($5x10^{-7}$ - $5x10^{-4}$ M), doses of atropine were adjusted for the steady state blood flow immediately prior to injection. Intracoronary injections of acetylcholine were used to determine the effectiveness of the muscarinic blockade. Low doses of

acetylcholine (1.0, 3.0 μg) were given prior to atropine, with increasing doses (1.0-100.0 μg) given following each increased concentration of atropine. The effects of atropine's vehicle, a 1% solution of methylparaben in distilled water, were tested by injecting the largest volume to be given (0.5 ml). Vehicle doses were given at least 20 minutes prior to the beginning of the atropine study. Responses in all parameters to acetylcholine were determined as the maximum change following injection.

Effect of Basal Muscarinic Blockade

Closed-chest Study (n=6)

The effect of resting cholinergic activity on basal coronary tone was investigated in the closed-chest sedated swine model. The electrocardiogram was not recorded since valium sedation did not provide adequate analgesia to use needle electrodes. Position of chronic thoracic catheters was verified prior to beginning the experiment by injection of radio-opaque dye (Renographin™,Squibb). Several minutes prior to each series of drug injections, position of the coronary catheters was verified by injection of radio-opaque dye.

Cholinergic influence on coronary blood flow was determined by intracoronary injection of the competitive muscarinic receptor blocking agent, atropine. One to five micrograms of acetylcholine, was injected as a test dose to determine the vascular response to muscarinic stimulation. Following muscarinic blockade with atropine (200 μg, i.c.), the acetylcholine test dose was repeated to determine the completeness of blockade. Abolition of hemodynamic changes induced by

the initial test dose indicated total blockade. Upon verification of muscarinic blockade, a second set of measurements was taken.

During a control period and following blockade, arterial and coronary sinus blood samples were withdrawn simultaneously into ice-cold heparinized glass syringes for determination of pH, pO2, pCO2 , oxygen content, and hematocrit. Following blood sampling, 15±3 μm carbonized plastic spheres labelled with a radioactive isotope were injected for measurement of regional coronary blood flow. The microspheres were sonicated at least 30 minutes prior to injection and thoroughly vortexed before injection. The microspheres were then injected into the left atrium to insure adequate mixing with the blood. An arterial blood sample was withdrawn (2.06 ml/min) beginning prior to, and continuing for at least one and a half minutes following microsphere injection. At the conclusion of the experiment, to identify myocardium receiving atropine, India ink dye was injected intracoronary immediately prior to flushing with a euthanasia solution, T-61, mixed with dye.

Effect of Basal Muscarinic Blockade

Pacing Study (n=10)

In these studies, heart rate was maintained constant through atrial-ventricular (A-V) pacing. The purpose of this pacing was to eliminate the changes in heart rate and pressure rate product which occurred in the closed-chest studies and which led to an increased myocardial oxygen consumption. The frequency of pacing was as close to the resting heart rate as possible while insuring maintenance of captured pacing. This frequency was 6-10 beats/min faster than resting

heart rate. The left atria and ventricle were stimulated with 5V for 1 ms. A-V delay was 100-150ms depending on heart rate. The square wave, single pulse stimulation was provided by a Grass S88 stimulator (Grass Medical Instruments, Quincy, Mass.)

Hemodynamic parameters were recorded, blood samples were taken, and microspheres were injected for flow determination as they were in the closed-chest study. Three sets of measurements were taken. The first was taken during a control period. The second following intracoronary injection of 1 mg (1mg/ml) of the beta- and alpha-adrenergic blocking agents, propranolol, and phentolamine, respectively [37]. The adequacy of alpha-adrenergic receptor blockade was tested by comparison of the flow response to bolus intracoronary injection of 20 μg phenylephrine (i.c.) before and after blockade. Comparison of the myocardial contractile response to 0.001-0.005 μg isoproterenol(i.c.) before and after blockade was used to determine the adequacy of beta-adrenergic blockade. Efficacy of cholinergic blockade was evaluated as in described in the previous section. Finally, the third measurement was taken following intracoronary injection of atropine (200 μg). The effects of muscarinic blockade on resting coronary flow were determined by comparison of the flow following muscarinic blockade to the flow following adrenergic blockade. Once again, India ink dye was injected intracoronary immediately prior to flushing with the euthanasia solution, T-61 mixed with dye, to identify myocardium receiving drugs.

Vagal Ligation Studies (n=6, n=3)

Following completion of the surgical preparation and a 30 minute

stabilization period, the nerves were reexposed. Heart rate was maintained constant with atrial-ventricular sequential pacing. (In three animals vagal ligation was performed without vagal ligation to provide an indication of parasympathetic activity to the heart.) First the right and then the left vagus nerve was ligated with two sutures and cut in between using a custom made glass "knife". Coronary blood flow and hemodynamic measurements were begun before and continued throughout the vagal ligation. Arterial and coronary sinus blood samples were drawn before and after complete vagal ligation. Responses in all parameters to vagal ligation were determined as the maximum change following injection.

DRUGS

Acetylcholine Bromide (Sigma) mixed with saline to 10 μg/ml, phentolamine (Regitine™, Ciba Pharmaceutical) mixed with distilled water to 1mg/ml, propranolol (Sigma) mixed with distilled water to 1 mg/ml, phenylephrine (Sigma) mixed with distilled water to 40 μg/ml, isoproterenol (Sigma) mixed with saline to 0.025 μg/ml, heparin (1000 USP units/ml, Elkins-Sinn Inc.), diazepam (Valium,5 mg/ml, Elkins-Sinn Inc.), atropine sulfate (400 μg/ml, Invenex Laboratories), ketamine (Vetalar™, 100 mg/ml, Parke-Davis), ampicillin (Polyflex™, 250 mg/ml, Bristol), isoflurane (Forane™, Anaquest), Renographin-76™ (Squibb), T-61 (American Hoechst Corp.), alpha-chloralose (Societe Chimique Pointet Girard) mixed with urethane (Sigma) and distilled water ((4.5 g chloralose/45 g urethane)/150 ml distilled water), lidocaine (Xylocaine™, Astra Pharmaceutical)

SAMPLE ANALYSIS

1.) Electromagnetic Flow Determination: Passage of charged ions through an electromagnetic field produces a proportional electrical flux. This electrical flux can be measured as millivolts. Components of blood carry electrical charges and thereby blood flow past the probe produces a voltage in the probe proportional to flow. A major charge carrying component of blood is red blood cells. Changes in hematocrit alter the voltages produced from a given flow. It is therefore necessary to adjust the flowmeter to account for hematocrit. Probes are calibrated at the factory and assigned a series of probe factors which allows for adjustment of the meter to various hematocrits. This calibration procedure involves perfusion of a vessel at various flow rates with blood of different hematocrits. The probe factor at a given hematocrit is adjusted so the meter agrees with the known flow rate.

On the day of the experiment, following probe placement and grounding of the meter to the animal, the flow meter was calibrated by; 1.) setting the probe factor to the animals measured hematocrit 2.) adjusting electric zero of the meter, 3.) switching the meter to null and adjusting the null knob until the minimum flow is reached, 4.) switching to balance and with the balance knob again adjusting to the minimum flow. The gain of the recording amplifier (Hewlett-Packard, 8802A) was adjusted to the desired level by output from the meter of a known constant voltage.

At the beginning and periodically throughout the experiment the electrical zero was checked against the actual zero by temporarily

occluding the vessel distal to the flow probe, with suture.

2.) Microsphere Flow Determination: Carbonized plastic beads of 15±3-micron diameter, labelled with the isotopes ^{141}Ce, ^{85}Sr, ^{46}Sc (3M, St. Paul, MN), ^{95}Nb, and ^{113}Sn, (New England Nuclear, Boston, MA) were used to determine regional myocardial blood flow as described by Heymann et al.[42]. Microspheres injected into the left atrium mix thoroughly with the blood as they pass into the ventricle and are ejected into the aorta [7]. The microspheres, although rheologically similar to red blood cells, are rigid and therefore become trapped as the blood passes through capillaries. Being evenly distributed within the blood, their entrapment within the capillaries is proportional to the blood flow. Therefore, by comparing the radioactivity in tissue samples to that of an arterial blood sample withdrawn at a known flow rate, the blood flow to tissue samples may be calculated.

The microspheres were stored in a saline suspension of 10% dextran including 0.05% polyoxyethylene 80 sorbitan monooleate (tween-80) to reduce aggregation. A volume of microspheres which had been calculated with respect to sample size, cardiac output, minimal final number of spheres/sample, and initial concentration, to yield at least $1x10^6$ spheres was withdrawn from the source vial. The amount of microsphere injected was sufficient to result in greater than 400 microspheres in the arterial withdrawal sample and in all tissue samples. Such a case will allow blood flow measurement with a 95% confidence limit [7,76].

Following saline dilution, the microspheres were sonicated for at least 30 minutes prior to injection. The microspheres were then

thoroughly vortexed before withdrawal into the injection syringe. Injection of the microspheres into the left atrium insured adequate mixing with the blood before entering the coronary circulation [7]. To minimize spheres remaining in the injection syringe, it was filled twice with saline which was then injected into the atrium. A syringe pump (Harvard Apparatus, 901) withdrew a reference arterial blood sample at a constant rate (2.06 ml/min) beginning prior to, and continuing for at least one and a half minutes following microsphere injection. The reference arterial blood sample and saline used to rinse the withdrawal sample syringe was then placed in tubes for subsequent counting of gamma radiation.

Following the experiment the heart and both kidneys were removed. After removing the atria and papillary muscles as well as extraneous connective tissue, the right and left ventricular free walls were separated from the septum. The left ventricle was then laid out flat and divided in grid fashion into four to five columns and rows. Each cube of left ventricle was then divided into 0.75±0.25 gram epi- and endo-cardial samples. The right ventricle and septum were then divided into five pieces each. For determination of flow, dyed left ventricular epi- and endo- cardial, right ventricular, and septal samples were pooled. A medial section of kidney was taken and connective tissue removed. All tissue pieces were weighed and placed into tubes for subsequent gamma-radiation counting (Tracor 2250, Elk Grove Village, IL). Record was kept of which tubes contain tissue pieces stained by the India ink dye injected at the time of death.

Each isotope used emits gamma-radiation at an easily

distinguishable characteristic energy level. Since more than one isotope was in any given piece of tissue, it was necessary to adjust for the appearance of higher energy emitting isotopes in the characteristic energy peak of the lower energy emitting isotopes. By counting pure samples of each isotope, it was possible to determine what proportion of each isotope appeared in each energy peak. Since the gamma spectrum of an isotope remains constant within a detection system, even in the presence of other isotopes, it was possible to subtract away the proportion of the higher energy isotope that appeared in any lower energy isotope energy peak.

Calculation of the true activity of each isotope when more than one isotope was present, used the equations of the stripping technique given below. The equation for subtraction of all higher energy isotopes is included below. When an isotope was not used it's factors were removed from the equation. All values were adjusted for background activity prior to use in these equations.

$$Ct(Sc) = Tcpm(Sc)$$

$$Ct(Nb) = Tcpm(Nb) - (Ct(Sc) \times Nb/Sc)$$

$$Ct(Sr) = Tcpm(Sr) - (Ct(Sc) \times Sr/Sc) - (Ct(Nb) \times Sr/Nb)$$

$$Ct(Sn) = Tcpm(Sn) - (Ct(Sc) \times Sn/Sc) - (Ct(Nb) \times Sn/Nb) - (Ct(Sr) \times Sn/Sr)$$

$$Ct(Ce) = Tcpm(Ce) - (Ct(Sc) \times Ce/Sc) - (Ct(Nb) \times Ce/Nb) - (Ct(Sr) \times Ce/Sr) - (Ct(Sn) \times Ce/Sn)$$

where Ct( ) = counts/min due solely to that isotope, Tcpm( ) = total counts/min appearing in that energy peak minus backround counts, and ratios such as Nb/Sc are proportionality factors determined from the

collected anaerobically in cold, heparinized glass syringes for determination of pO2, pCO2, pH, and oxygen content. The OSM3 determines oxygen content by measuring the amount of oxyhemoglobin in a hemolyzed blood sample at six different wave-lengths and multiplying by the oxygen carrying capacity value, 1.39 $ml O_2/g$ hemoglobin. Arterial and venous hematocrits were also be determined from these samples. The blood was mixed prior to blood gas and hematocrit determination.

Arterial venous oxygen content difference or the oxygen extraction was derived by subtracting coronary venous from arterial oxygen content. This arteriovenous oxygen content difference multiplied by the total flow to the left ventricular free wall yielded average myocardial oxygen consumption ($ml O_2/min/100g$) according to the Fick equation. The arteriovenous oxygen content difference was divided by the arterial oxygen content to yield the percent oxygen extraction.

3.) Pressure rate product was determined by multiplying peak systolic pressure by heart rate.

DATA ANALYSIS

Data was collected during control and at the peak of the respective response and the absolute change analyzed for significance. The organization of the data was such that all responses were controlled within the same animal, therefore the Student's t-test for paired samples was used to analyze all data. Linear relationships between parameters were analyzed by linear regression. Significant difference

from maximal volume vehicle injection (1.0 ml saline) was determined for each drug dose in the acetylcholine study, using an analysis of variance for a completely randomized design followed by a Duncan multiple range test [16]. Statistical tests were performed on a Hewlett-Packard 1000 computer with customized programs. Differences between data were considered to be significant when $p < 0.05$. Parameters which did not change significantly, were analyzed for the probability of falsely accepting the null hypothesis, or beta error. Beta error (β) was determined from the z statistic;

$$z = \frac{X - \mu}{\sigma / \sqrt{n}}$$

where X = sample mean, μ = population mean, σ^2 = sample variance, and n = number in sample. The area under the upper tail of the normal curve corresponding to the calculated z statistic equalled the beta error. The power of the study equalled $1 - \beta$.

RESULTS

Acetylcholine Study

Flow and Resistance data

Left anterior descending coronary artery blood flow and resistance data, prior to and following intracoronary doses of acetylcholine, are illustrated in Figure 6. Blood flow was significantly reduced in a dose dependent manner by acetylcholine doses in the range of 0.25 to 3.0 μg. Vascular resistance was significantly increased over the same range with the exceptions of 0.75, 1.0, and 3.0 μg doses.

There were no significant differences among the control coronary blood flows which averaged 167.1 ± 3.12 ml/min/100g. The greatest average reduction from this mean was 77% caused by 2.5 μg acetylcholine. Control coronary vascular resistances also had no significant differences. The mean control value of 0.588 ± 0.01 mmHg/ml/min/100g was increased 370% by 2.5 μg acetylcholine.

Hemodynamic data

The relationship between the coronary blood flow and hemodynamic changes that occur following a saline flush (1.0 ml), and a low (0.5μg) and high dose (2.0 μg) of acetylcholine, before and after atropine, can be seen in Figure 7. Phasic and mean coronary flow are dramatically reduced while the reductions in dP/dt, intraventricular and mean

arterial pressure are relatively small. The mean percent change in flow following 2.0 μg of acetylcholine was 70% while that of intraventricular and mean arterial pressure was only 5.1% and 7.7%, respectively. Acetylcholine has no effect on any of these parameters following atropine.

Summarized in Table I are the hemodynamic data obtained prior to and following intracoronary injection of various doses of acetylcholine. Significant changes from control saline injections, in mean arterial pressure, occurred following injection of 1.0, 1.5 through 3.0 μg doses of acetylcholine. The remainder of the dosages produced no significant changes in mean arterial pressure. Peak systolic pressure was significantly reduced by doses of acetylcholine greater than 0.75 μg. Neither heart rate nor pressure rate product were significantly changed by any dose of acetylcholine. Finally, dP/dt was significantly reduced by acetylcholine doses of 0.5 μg and greater. Following muscarinic receptor blockade, acetylcholine (0.25 to 3 μg) had no effect on any hemodynamic parameter.

The atropine-induced attenuation of coronary blood flow changes in response to acetylcholine is illustrated in Figure 8. Reductions in coronary blood flow and vascular resistance caused by acetylcholine were greatly attenuated by the intracoronary injection of 200 μg atropine. Comparatively small reductions in coronary blood flow were achieved with large doses (30 μg) of acetylcholine, indicating that the blockade could be overcome with agonist. Systemic effects, such as decreased arterial pressure or heart rate, did not occur at the higher doses of acetylcholine.

Atropine dose study

Table III lists the mean percent reduction in coronary blood flow following increasing doses of acetylcholine given before and after additive molar log doses of atropine. Low doses of acetylcholine given prior to atropine produced substantial reductions in coronary blood flow. These reductions were blunted but not abolished by a low dose of atropine, 5×10^{-7} M. Greater doses of atropine provided a greater blocking effect and allowed administration of greater doses of acetylcholine. The highest dose of atropine, approximating 100 μg, blocked doses of 30 μg or less and greatly attenuated acetylcholine doses of 50 and 100 μg. No dose of atropine produced any changes in the hemodynamic parameters such as heart rate, pressure rate product, mean arterial or peak intraventricular pressure, or dP/dt.

Atropine doses of 100 μg injected into the left anterior descending coronary artery adequately blocked flow responses to acetylcholine. With the assumption that blood flow to the whole ventricle could be twice that of the left anterior descending coronary artery, 200 μg was chosen as the dose of atropine to be used in subsequent studies of whole left ventricular flow. The vehicle for atropine (a 1% solution of methylparaben dissolved in distilled water), produced only a transient hyperemia, but had no effect on the coronary vascular responses produced by intracoronary acetylcholine. A 2.0 μg dose of acetylcholine reduced coronary flow 69.2 ± 9.1 % before, and 69.9 ± 8.4 % following 0.5 ml of 1% methylparaben (n=3).

Closed-chest, sedated studies

Flow and resistance data

Whole left ventricular flow and resistance data obtained during this study of valium sedated closed-chest swine is given in Table IV. In these studies, coronary blood flow was significantly increased 18.25%, from a control 286.0 ± 45.4 to 338.2 ± 48.4 ml/min/100g, following intracoronary injection of atropine (200 μg). Concomitantly, coronary vascular resistance was significantly reduced 14.75% from a control 0.400 ± .046 to 0.341 ± .03 mmHg/ml/min/100g.

Regional myocardial blood flow and resistance are also depicted in Table IV. Both epicardial and endocardial blood flows increased significantly as their respective resistances decreased. The increase in endocardial flow was less than epicardial flow, thereby resulting in an endocardial/epicardial blood flow ratio which fell slightly (5.4%) yet significantly from 1.30 ± .037 to 1.23 ± .026. Regional vascular resistance was significantly reduced in the epicardial region, however the decrease was not significant in the endocardium.

Hemodynamic data

Coronary blood flow and resistance changes were accompanied by significant changes in heart rate and pressure rate product, as presented in Table V. Heart rate increased 20.2% while pressure rate product increased 20.7%. Coronary blood flow and resistance are related to heart rate and pressure rate product as illustrated in Figures 9 and 10, respectively. Individual points are plotted for control and

response for each animal (n=6) as well as the means ± standard error of the means for each group. There was not a statistical difference in the regression lines for control and following atropine for either heart rate or pressure rate product. Therefore, all points for each graph were used to calculate the linear regression line. Heart rate correlated significantly with coronary blood flow ($r=0.81$, $p< 0.05$) and vascular resistance($r=0.79$, $p <0.05$). Increases in pressure rate product were also significantly related to increases in flow ($r=0.92$, $p <0.05$) and decreases in resistance ($r=0.77$, $p <0.05$). There were no significant differences from control in mean arterial or peak systolic pressure following atropine.

Oxygen Content, Consumption and Delivery data

The data in Table VI are from those parameters related to the oxygen content of the blood. Blood gas data is presented in Table VII. Myocardial oxygen consumption increased significantly 25.4 % following atropine, from a control 18.5 ± 2.0 to 23.2 ± 3.0 $ml O_2/min/100g$. The arterial oxygen content also significantly increased likely contributing to the significant increase in myocardial oxygen delivery. Oxygen extraction did not increase with the increased arterial oxygen content. This led to a slight, however not significant, increase in coronary sinus oxygen content. The significant relationship of coronary blood flow ($r=0.57$, $p< 0.05$) and resistance with myocardial oxygen consumption ($r=0.59$, $p< 0.05$) is illustrated in Figure 11. Once again, since there was no statistical difference between the regression lines of control and following atropine, the two groups were combined to yield the

calculated regression line.

Paced studies

Flow and resistance data

When heart rate and pressure rate product were held constant by atrial-ventricular sequential pacing and adrenergic blockade, whole left ventricular coronary blood flow and vascular resistance did not change significantly following injection of atropine (Table VIII). These studies had the statistical power to detect significant changes in flow and resistance as significant with 83 and 90 percent confidence, respectively.

Regional myocardial blood flows and resistances, included in Table VIII, also did not change significantly following atropine. However, there was again a slight yet significant decrease in the endocardial/epicardial blood flow ratio, apparently due to a slightly greater reduction in endocardial flow than epicardial flow.

Hemodynamic data

The hemodynamic data of the paced study are presented in Table IX. Heart rate was maintained constant through atrial-ventricular pacing and therefore did not change with atropine. Prior administration of adrenergic blocking agents prevented any changes in peak systolic or mean arterial pressure. Since heart rate and peak systolic pressure did not change, their product, pressure rate product, did not significantly change either. However, there was a significant increase of 27.5% in the first derivative of pressure, dP/dt.

Oxygen Content, Consumption and Delivery data

Data derived from oxygen content are presented in Table X. (Blood gas data presented separately in Table XI) Myocardial oxygen consumption did not change significantly, nor did oxygen delivery and arterial oxygen content. The lack of change in these parameters when compared to the closed-chest study reflects the effects of pacing and prior adrenergic blockade in maintaining a steady state of myocardial function. As with the closed-chest study there were no significant changes in venous oxygen content, oxygen extraction, or % oxygen extraction.

The significant relationship of coronary blood flow ($r=0.66$, $p< 0.05$) and resistance ($r=0.46$, $p< 0.05$), with myocardial oxygen consumption is graphed in Figure 12. The regression line was calculated from data combined from before and following atropine, since there was no statistical difference between their individual regression lines.

All observations from the closed-chest sedated and open-chest paced studies were used to calculate the linear regression line presented in Figure 13. When the mean values from control and following atropine were plotted, they fell along the regression line. It is evident that changes in myocardial oxygen consumption following atropine during the closed-chest study resulted in the increased flow and decreased resistance. Conversely, when myocardial oxygen consumption was held constant in the paced studies, there were no significant changes in flow and resistance following atropine.

Vagal ligation study

Flow and resistance data

Controls and right and left vagal ligation coronary blood flow and resistance data from paced animals are given in Table XII. There were no significant changes in either coronary blood flow or resistance following either right or left vagal ligation. Also, there was no significant difference in the flow or resistance after ligation of both vagi as compared to the control prior to any manipulation. These studies had at least an 80% power to determine the changes in flow and resistance which occurred as significant.

In three nonpaced animals, right and left vagal ligation was performed to determine the extent of basal vagal activity to the heart by evaluating the influence on heart rate. Heart rate increased approximately 10% following right and left vagal ligation, from averages of 135 ± 15 to 146 ± 14 and 142 ± 7 to 158 ± 5, respectively. The increase in heart rate following bilateral ligation was approximately 17%.

Hemodynamic data

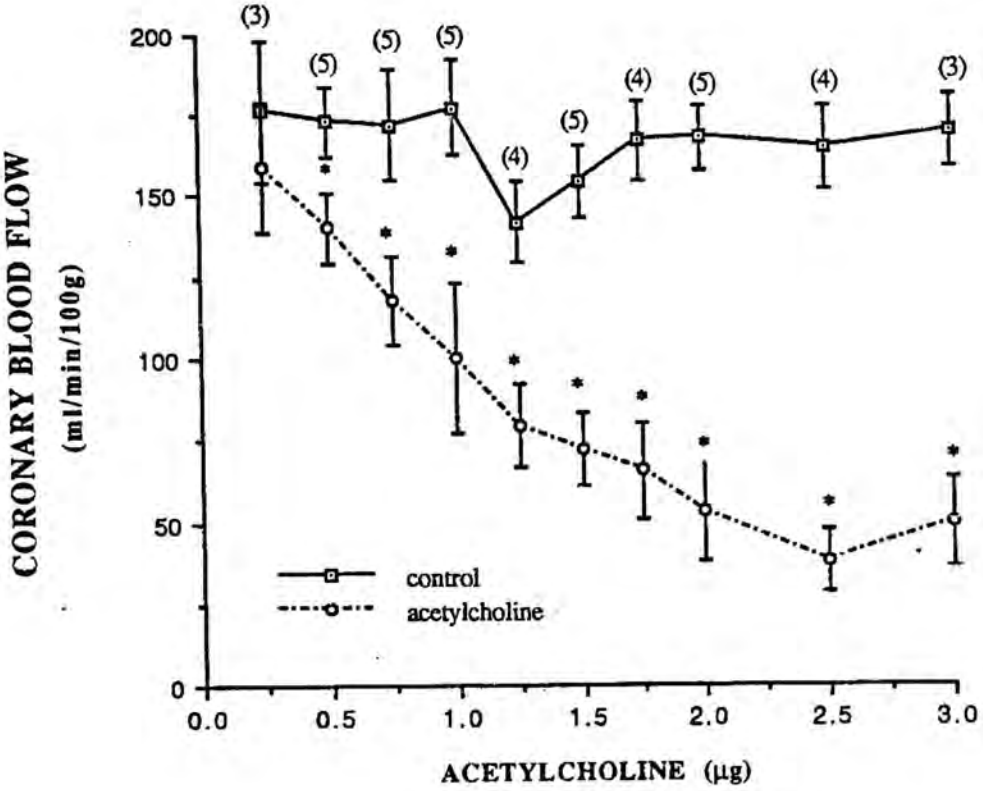
Hemodynamic data during the control periods and following right and left vagal ligation are presented in Table XIII. Following either right or left vagal ligation, heart rate, mean arterial and peak systolic pressure, pressure rate product and dP/dt did not change significantly. These studies were able to detect a change of 5% as significantly different with a power of at least 80%.

Oxygen Content, Consumption and Delivery data

Consistent with the results from the Basal Effects of Atropine - Closed-Chest Study - when myocardial oxygen consumption remained constant following vagal ligation, neither coronary sinus oxygen content nor oxygen extraction changed significantly (Table XIV). (Blood gas data presented separately in Table XV)

Blood gas parameters for all studies were comparable to previously reported values [33].

Figure 6. Coronary blood flow and resistance changes following intracoronary doses of acetylcholine ranging from 0.25 μg to 3.0 μg. Control (——) and peak response (- - - -) values are mean ± S.E.M. (#) indicates the number of animals to which each dose was given. Response values significantly different from control are indicated by *. Changes in coronary blood flow and vascular resistance induced by acetylcholine were statistically compared to changes occurring after equal volume injections of saline as control for significance by the Duncan's multiple range test at the $p<0.05$ level. Control values for blood flow and resistance did not vary significantly as determined by Duncan's multiple range test.


CORONARY BLOOD FLOW
(ml/min/100g)
200
150
100
50
0
(3)
(5)
(5)
(5)
(4)
(5)
(4)
(5)
(4)
(3)
control
acetylcholine
0.0
0.5
1.0
1.5
2.0
2.5
3.0
ACETYLCHOLINE (µg)

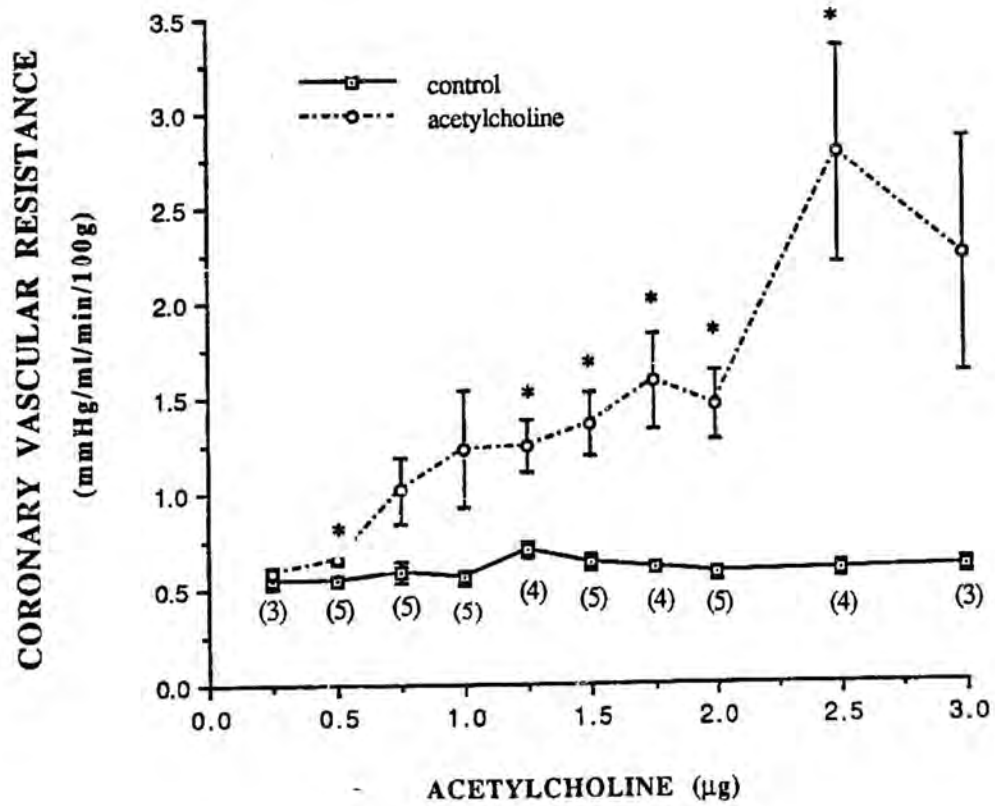

CORONARY VASCULAR RESISTANCE
(mmHg/ml/min/100g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
control
acetylcholine
(3)
(5)
(5)
(5)
(4)
(5)
(4)
(5)
(4)
(3)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
ACETYLCHOLINE (µg)

Figure 7. Hemodynamic tracings of dP/dt, intraventricular and mean arterial pressure, and phasic and mean coronary blood flow illustrate the changes produced by intracoronary injection a 1 ml saline flush, 0.5 and 2.0 μg acetylcholine and 2.0 μg acetylcholine following atropine (200 μg, i.c.).

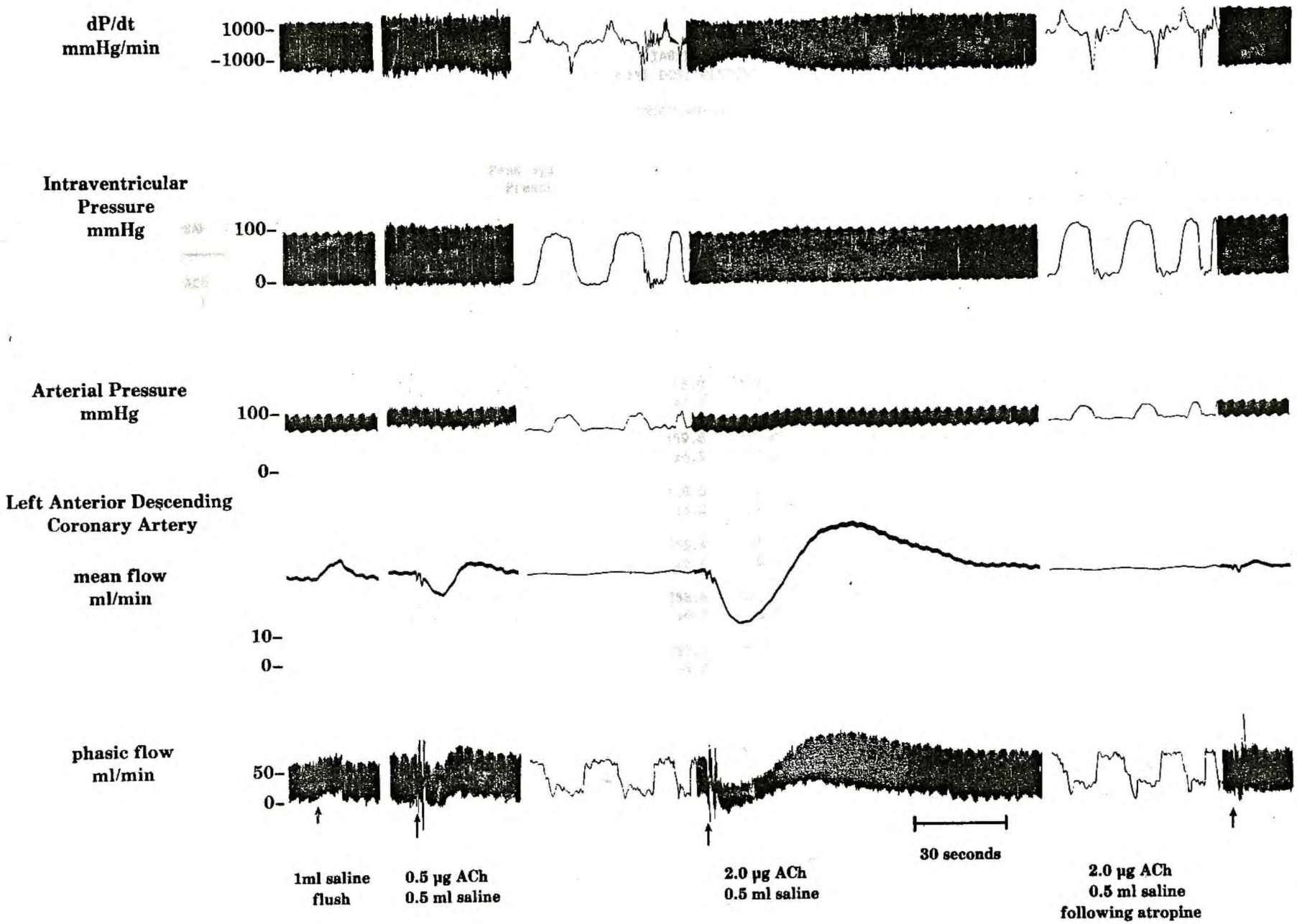

dP/dt
mmHg/min
1000-
-1000-
Intraventricular
Pressure
mmHg
100-
0-
Arterial Pressure
mmHg
100-
0-
Left Anterior Descending
Coronary Artery
mean flow
ml/min
10-
0-
phasic flow
ml/min
50-
0-
30 seconds
1ml saline
flush
0.5 µg ACh
0.5 ml saline
2.0 µg ACh
0.5 ml saline
2.0 µg ACh
0.5 ml saline
following atropine

TABLE I
ACETYLCHOLINE DOSE RESPONSE STUDY

HEMODYNAMICS

SAMPLE	Mean Arterial Blood Pressure mmHg C	R	Peak Systolic Pressure mmHg C	R	Heart Rate beats/min C	R	Pressure Rate Product mmHg/min C	R	dP/dt mmHg/min C	R
ACh 0.25 µg	93.0	91.3	114.0	114.0	157.5	150.0	17887.5	16965.0	2350	2100
n=2	±5.7	±6.8	±6.4	±6.4	±5.3	±10.6	±397.7	±254.6	±460	±283
ACh 0.5 µg	93.3	90.2	114.6	111.4	155.6	157.0	17881.0	17559.0	2515	2169 *
n=5	±4.2	±4.9	±4.7	±4.9	±6.1	±5.8	±1147.7	±1208.0	±415	±348
ACh 0.75 µg	96.3	93.9	119.0	115.0	158.0	159.0	18826.0	18313.0	2500	2230 *
n=5	±3.5	±3.8	±3.6	±3.9	±7.3	±7.1	±1112.4	±1134.9	±374	±380
ACh 1.0 µg	96.1	90.2 *	119.0	112.6 *	159.8	158.0	19033.0	17836.0	2750	2375 *
n=5	±4.4	±5.8	±3.8	±4.8	±6.5	±7.3	±1046.2	±1288.9	±424	±370
ACh 1.25 µg	96.6	91.3	119.3	114.3 *	160.0	162.5	19063.3	18620.0	2375	1831 *
n=4	±4.2	±4.9	±4.3	±5.1	±5.8	±7.2	±923.1	±1427.9	±472	±338
ACh 1.5 µg	95.6	89.7 *	118.0	111.0 *	153.4	153.0	18175.0	17055.0	2437	1875 *
n=5	±4.6	±5.8	±5.4	±6.5	±6.4	±5.8	±1360.6	±1410.3	±391	±296
ACh 1.75 µg	79.5	72.1 *	124.5	114.5 *	158.0	161.3	19636.0	18472.5	2417	1942 *
n=4	±18.2	±16.8	±4.4	±6.1	±6.7	±8.2	±956.5	±1460.0	±340	±339
ACh 2.0 µg	93.9	86.7 *	116.0	110.2 *	152.8	154.0	17823.0	17060.0	2594	1988 *
n=5	±5.1	±5.2	±6.2	±6.5	±5.8	±6.2	±1443.7	±1447.8	±462	±347
ACh 2.5 µg	76.1	69.8 *	115.8	108.3 *	148.8	147.5	17265.0	15995.0	2515	1781 *
n=4	±17.3	±15.9	±4.7	±5.0	±6.3	±5.2	±1234.8	±1085.7	±386	±251
ACh 3.0 µg	101.2	90.9 *	125.0	113.3 *	156.3	161.7	19536.7	18450.0	2733	1900 *
n=3	±5.2	±7.0	±7.1	±8.3	±6.7	±10.6	±1405.9	±2296.0	±628	±449

Values are mean ± S.E.M.
C = values taken during control
R = values taken during maximum change from control
* indicates significantly different from saline control injection at $p < 0.05$

Figure 8. Percent reduction in coronary blood flow produced by acetylcholine before (——)(n=43) and after (— - - —)(n=10) atropine (200 μg, intracoronary).

TABLE II

BLOOD GASES:

ACETYLCHOLINE DOSE RESPONSE STUDY

	pO2	pCO2	pH	HEMATOCRIT
arterial	87.03 ±4.11	33.23 ±1.57	7.50 ±0.02	32.54 ±1.32
venous	25.97 ±2.65	42.70 ±2.17	7.45 ±0.03	34.80 ±0.57

Values are means ± S.E.M.

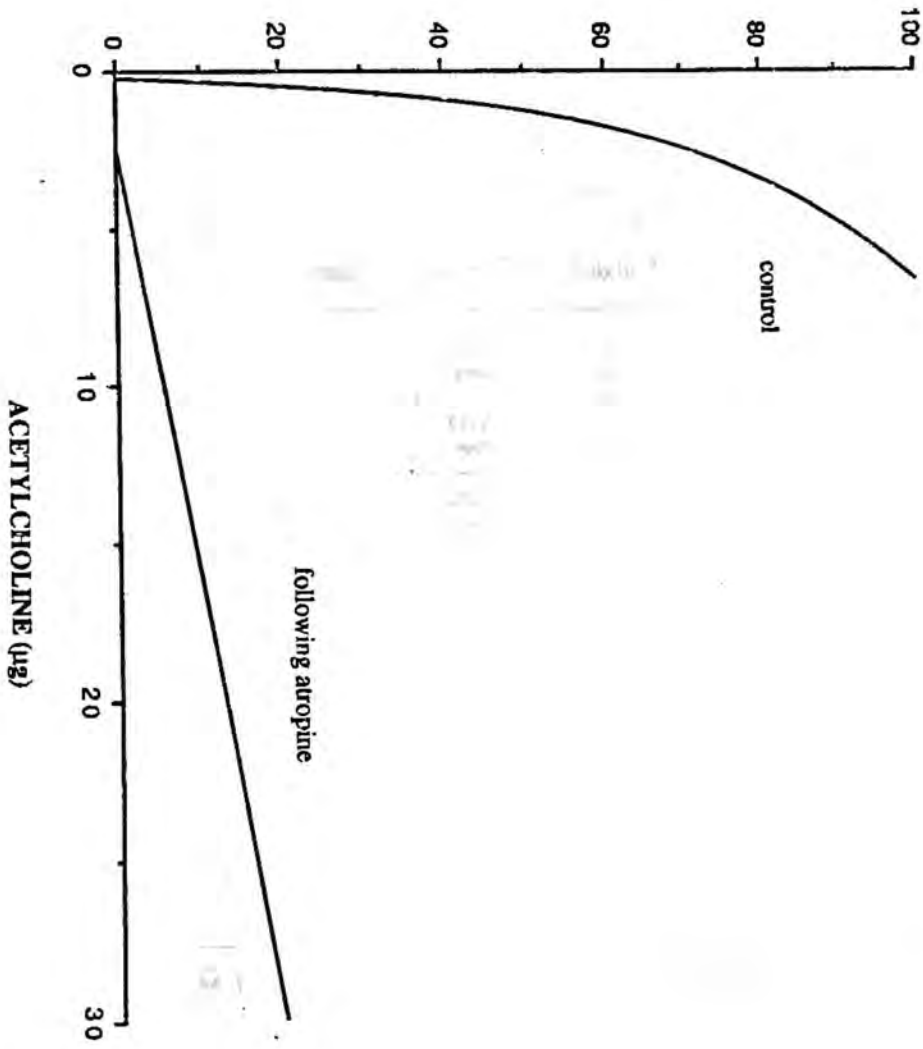

% REDUCTION IN CORONARY BLOOD FLOW
0
20
40
60
80
100
control
following atropine
0
10
20
30
ACETYLCHOLINE (µg)

TABLE III

ATROPINE DOSE RESPONSE STUDY

% REDUCTION LEFT ANTERIOR DESCENDING
DESCENDING CORONARY BLOOD FLOW

ACh DOSE		ATROPINE CONCENTRATION (M)			
μg	CONTROL	$5.0x10^{-7}$	$5.0x10^{-6}$	$5.0x10^{-5}$	$5.0x10^{-4}$
1	49 n=4	21.35 n=4	0 n=4	**	**
3	50.6 n=2	31.7 n=3	5.6 n=4	**	**
5		20.7 n=2	14.05 n=5	**	**
10			36 n=3	7.45 n=2	**
20			10.5 n=1	7.7 n=2	**
30			29.7 n=1	11.7 n=4	2.4 n=3
50				24.7 n=3	7.8 n=3
100					11.05 n=2

** indicates doses not given, assumed not to have effect

TABLE IV

BASAL EFFECTS OF MUSCARINIC BLOCKADE : CLOSED-CHEST STUDY

WHOLE AND REGIONAL LEFT VENTRICULAR

CORONARY BLOOD FLOW AND RESISTANCE DATA

Panel A

Whole
Left Ventricular

	Flow ml/min/100g	Resistance mm/ml/min/100g
CONTROL n=6	286.0 ±45.4	0.400 ±0.046
ATROPINE n=6	338.2 * ±48.4	0.341 * ±0.030

Panel B

Regional
Left Ventricular

	Epicardial			Endocardial	
	Flow ml/min/100g	Resistance mmHg/ml/min/100g	Endo/Epi Flow Ratio	Flow ml/min/100g	Resistance mmHg/ml/min/100g
CONTROL n=6	252.8 ±37.1	0.485 ±0.071	1.30 ±0.04	326.1 ±51.0	0.355 ±0.041
ATROPINE n=6	307.0 * ±44.7	0.409 * ±0.053	1.23 * ±0.03	375.9 * ±52.8	0.307 ±0.027

Values are mean ± S.E.M.
* indicates values significantly different from control at $p < 0.05$.

TABLE V

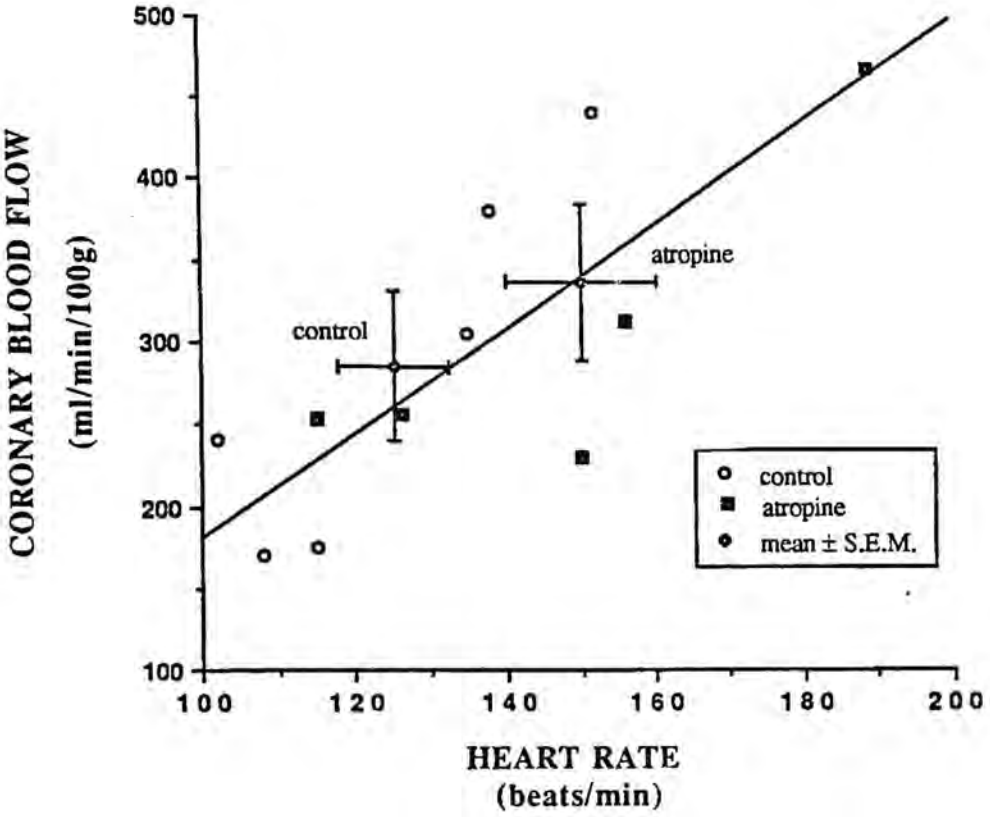
BASAL EFFECTS OF MUSCARINIC BLOCKADE : CLOSED-CHEST STUDY

HEMODYNAMICS

	Heart Rate beats/min	Blood Pressure mmHg	Peak Systolic Pressure mmHg	Pressure Rate Product mmHg/min
CONTROL n=6	125.0 ±7.3	104.0 ±5.7	126.8 ±6.7	16061.5 ±1708.8
ATROPINE n=6	150.2 * ±10.0	106.1 ±6.8	127.3 ±8.9	19392.2 * ±2236.0

Values are mean ± S.E.M.
* indicates values significantly different from control at $p < 0.05$

Figure 9. Coronary blood flow and vascular resistance are plotted with respect to heart rate. Individual points are plotted for control (■) and following intracoronary injection of 200 μg atropine (O). Means() ± standard error of the means (S.E.M.) before and after atropine are also presented. Linear regression performed for each group yield regression lines which were not statistically different. Therefore, the calculated linear regression line presented represents the combined data for both groups. The relationship between coronary blood flow and heart rate ($r=0.81$, $m=3.54$, y-intercept=-173.9) was significant ($p< 0.05$) as was the relationship between vascular resistance and heart rate($r=0.79$, $m=-2.94$, y-intercept=0.77, $p< 0.05$).


CORONARY BLOOD FLOW
(ml/min/100g)
500
400
300
200
100
100
120
140
160
180
200
HEART RATE
(beats/min)
control
atropine
control
atropine
mean ± S.E.M.

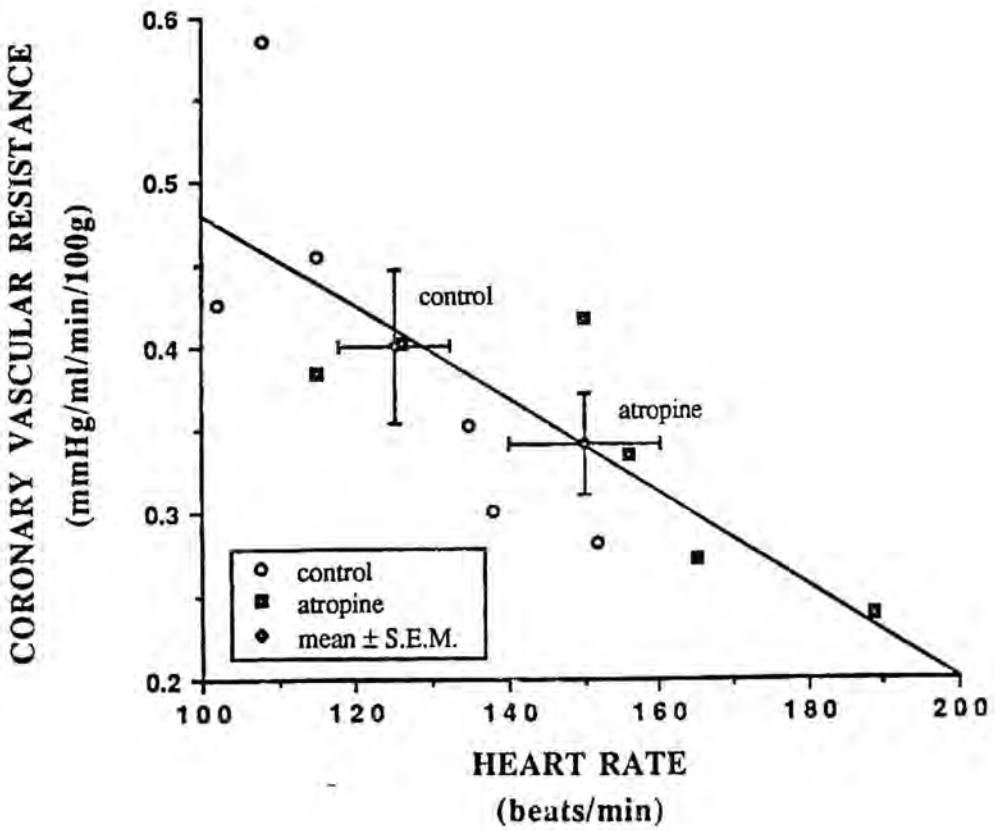
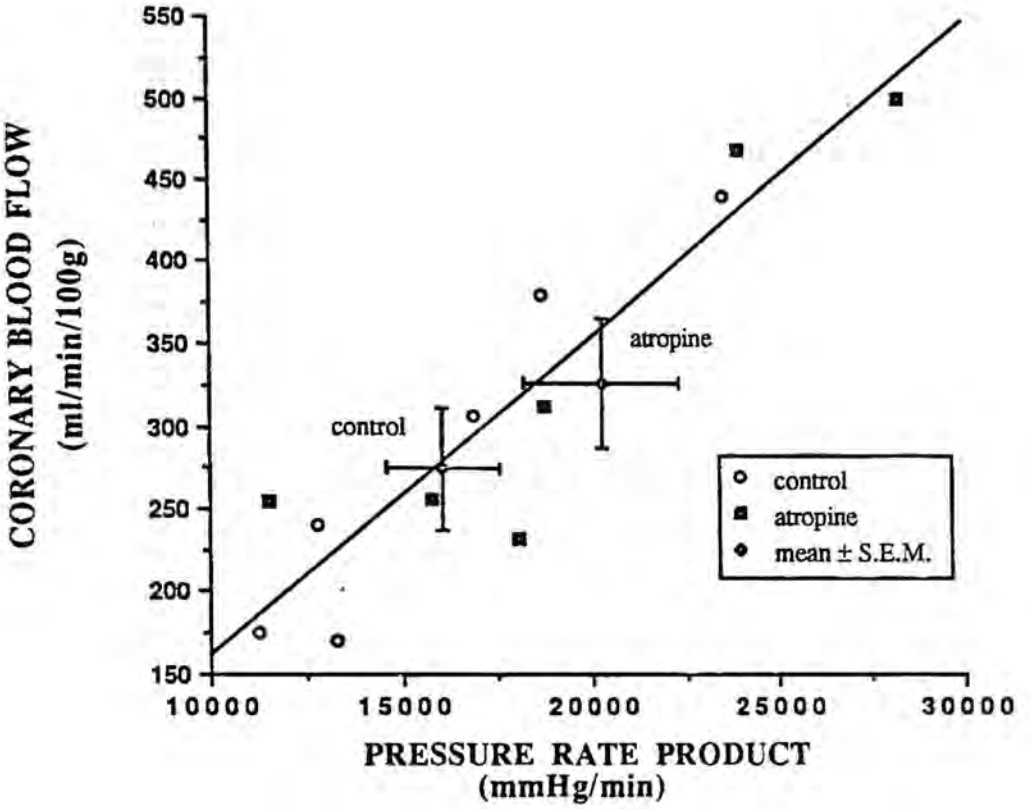

CORONARY VASCULAR RESISTANCE
(mmHg/ml/min/100g)
0.6
0.5
0.4
0.3
0.2
100
120
140
160
180
200
HEART RATE
(beats/min)
control
atropine
control
atropine
mean ± S.E.M.

Figure 10. Coronary blood flow and vascular resistance are plotted with respect to pressure rate product. Individual points are plotted for control (■) and following intracoronary injection of 200 μg atropine (○). Means( ) ± S.E.M. before and after atropine are also presented. Linear regression performed for each group yield regression lines which were not statistically different. Therefore, the calculated linear regression line presented represents the combined data for both groups. The relationship between coronary blood flow and pressure rate product ($r=0.92$, $m=0.02$, y-intercept=-32.75) was significant ($p< 0.05$) as was the relationship between vascular resistance and pressure rate product ($r=0.77$, $m=1.4\times10^{-5}$, y-intercept=0.62, $p< 0.05$).


CORONARY BLOOD FLOW
(ml/min/100g)
550
500
450
400
350
300
250
200
150
10000
15000
20000
25000
30000
PRESSURE RATE PRODUCT
(mmHg/min)
control
atropine
control
atropine
mean ± S.E.M.

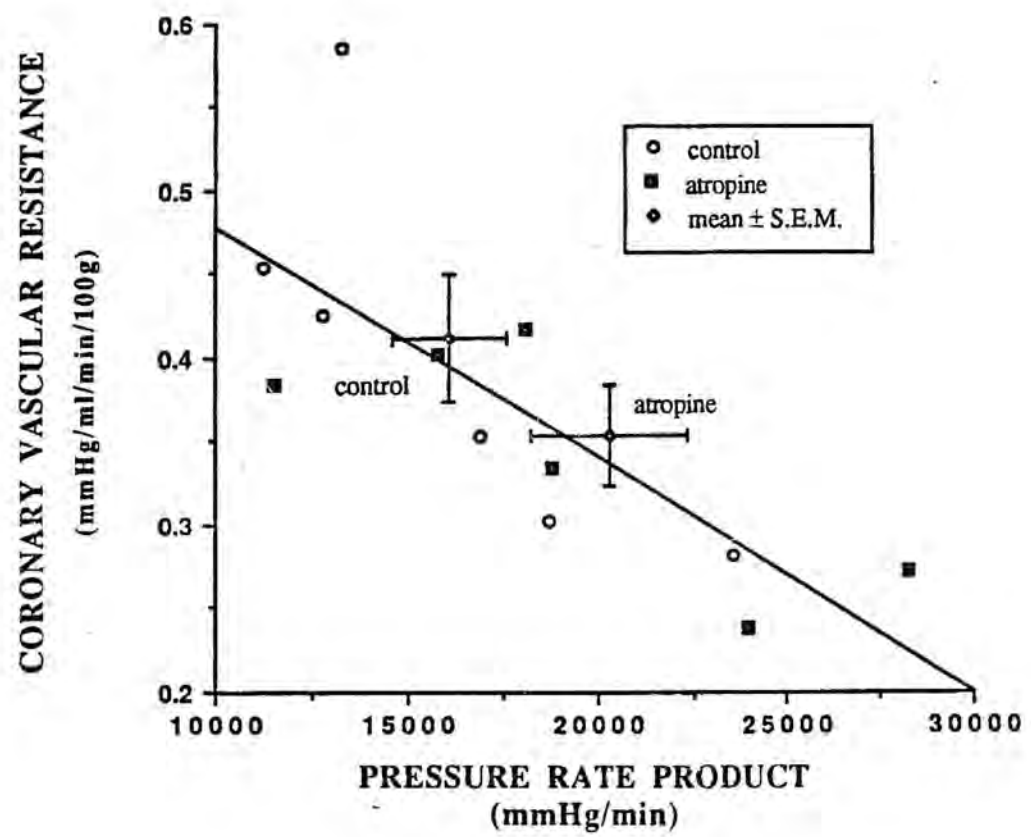

CORONARY VASCULAR RESISTANCE
(mmHg/ml/min/100g)
0.6
0.5
0.4
0.3
0.2
10000
15000
20000
25000
30000
PRESSURE RATE PRODUCT
(mmHg/min)
control
atropine
mean ± S.E.M.
control
atropine

TABLE VI

BASAL EFFECTS OF MUSCARINIC BLOCKADE : CLOSED-CHEST STUDY

OXYGEN CONTENT AND DERIVED VALUES

Left Ventricular

	Oxygen Content			%O2 extraction	Consumption	Delivery
	Arterial	Venous	A-V			
	$ml O_2$/100ml blood				$ml O_2$/min/100g	
CONTROL n=6	9.7 ±0.7	2.7 ±0.6	7.0 ±0.9	71.80% ±6.27%	18.5 ±2.0	26.2 ±2.4
ATROPINE n=6	10.3 * ±0.8	3.1 ±0.6	7.2 ±0.8	69.27% ±5.96%	23.2 * ±3.0	33.3 * ±3.2

Values are mean ± S.E.M.
* indicates values significantly different from control at $p < 0.05$

TABLE VII

BASAL EFFECTS OF MUSCARINIC BLOCKADE

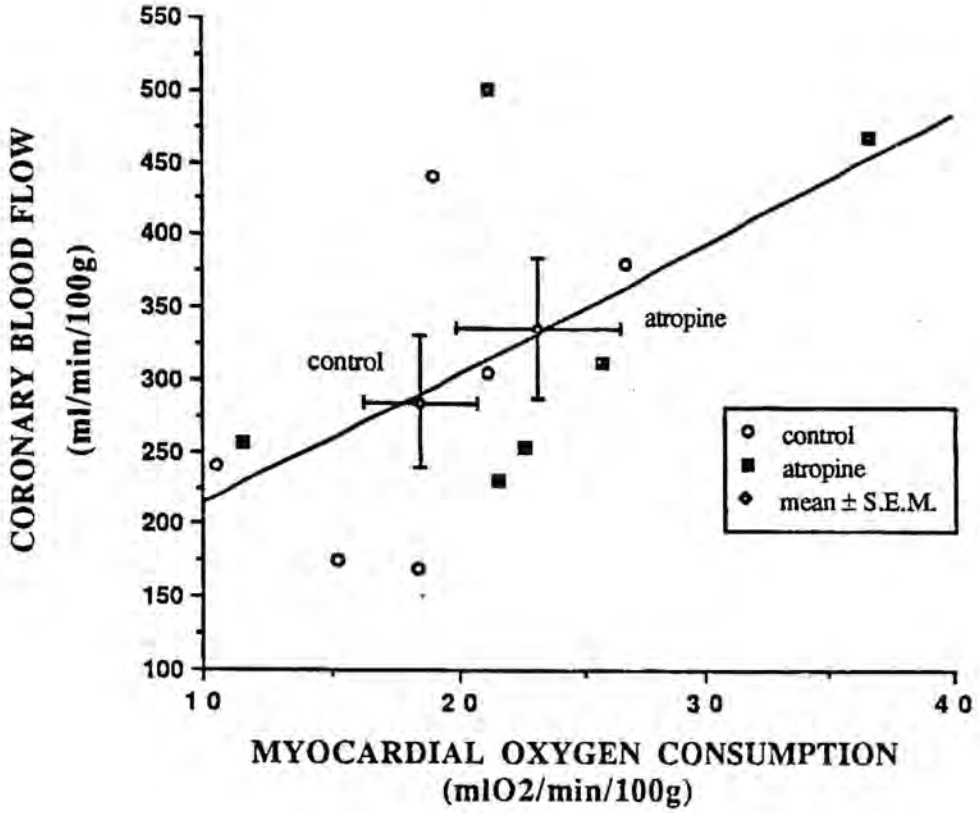
BLOOD GASES

CLOSED-CHEST STUDIES

	pO2		pCO2		pH		HEMATOCRIT %	
	A	V	A	V	A	V	A	V
CONTROL	75.72 ±3.58	21.63 ±5.77	45.08 ±2.48	46.03 ±9.71	7.40 ±0.03	6.17 ±1.23	21.9 ±4.4	21.6 ±4.3
ATROPINE	75.20 ±5.18	21.05 ±5.37	43.67 ±2.60	43.82 ±9.35	7.42 ±0.03	6.19 ±1.24	23.3 ±3.7	23.3 ±2.8

Values are means ± S.E.M.

Figure 11. Coronary blood flow and vascular resistance are plotted with respect to myocardial oxygen consumption. Individual points are plotted for control (■) and following intracoronary injection of 200 μg atropine (○). Means ± S.E.M. before and after atropine are also presented. Linear regression performed for each group yield regression lines which were not statistically different. Therefore, the calculated linear regression line presented represents the combined data for both groups. The relationship between coronary blood flow and myocardial oxygen consumption ($r=0.57$, $m=9.1$, y-intercept=122.9) was significant ($p< 0.05$) as was the relationship between vascular resistance and myocardial oxygen consumption ($r=0.59$, $m=8.0\times10^{-3}$, y-intercept=0 .54, $p< 0.05$).


CORONARY BLOOD FLOW
(ml/min/100g)
550
500
450
400
350
300
250
200
150
100
10
20
30
40
control
atropine
control
atropine
mean ± S.E.M.
MYOCARDIAL OXYGEN CONSUMPTION
(mlO2/min/100g)

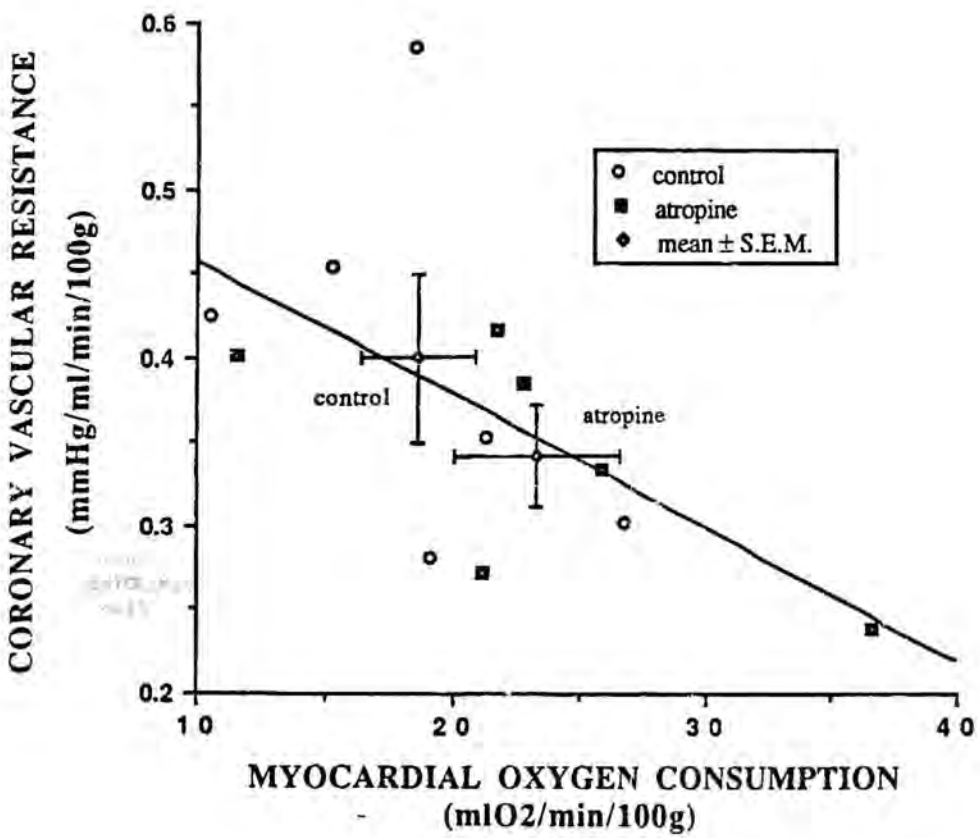

CORONARY VASCULAR RESISTANCE
(mmHg/ml/min/100g)
0.6
0.5
0.4
0.3
0.2
10
20
30
40
control
atropine
control
atropine
mean ± S.E.M.
MYOCARDIAL OXYGEN CONSUMPTION
(mlO2/min/100g)

TABLE VIII

BASAL EFFECTS OF MUSCARINIC BLOCKADE : PACED STUDIES

WHOLE AND REGIONAL LEFT VENTRICULAR

CORONARY BLOOD FLOW AND RESISTANCE

Whole Left Ventricular

	Flow ml/min/100g	Resistance mmHg/ml/min/100g
CONTROL n=10	138.0 ±19.6	0.749 ±0.117
PROPRANOLOL PHENTOLAMINE n=10	125.3 ±13.8	0.671 ±0.060
ATROPINE n=10	121.7 ±14.5	0.714 ±0.079

Regional Left Ventricular

	Epicardial		Endo/Epi Flow	Endocardial	
	Flow ml/min/100g	Resistance mmHg/ml/min/100g		Flow ml/min/100g	Resistance mmHg/ml/min/100g
CONTROL n=10	132.2 ±19.1	0.753 ±0.132	1.090 ±0.044	142.5 ±20.4	0.710 ±0.110
PROPRANOLOL PHENTOLAMINE n=10	120.6 ±39.6	0.745 ±0.192	1.090 ±0.111	130.7 ±44.6	0.663 ±0.173
ATROPINE n=10	119.5 ±42.5	0.737 ±0.262	1.046 * ±0.120	124.3 ±46.3	0.702 ±0.233

Values are mean ± S.E.M.
* indicates values significantly different from appropriate control values at $p < 0.05$.

TABLE IX

BASAL EFFECTS OF MUSCARINIC BLOCKADE : PACING STUDIES

HEMODYNAMICS

	Heart Rate beats/min	Blood Pressure mmHg	Peak Systolic Pressure mmHg	Pressure Rate Product mmHg/min	dP/dt mmHg/min
CONTROL n=10	165.8 ±3.4	88.7 ±3.8	103.6 ±4.2	17163.3 ±766.1	1799.1 ±109.5
PROPRANOLOL PHENTOLAMINE n=10	166.3 ±3.7	78.3 ±3.1	91.0 ±3.5	15094.1 ±552.6	1206.3 ±96.9
ATROPINE n=10	166.5 ±3.7	79.5 ±4.9	92.6 ±5.3	15378.5 ±887.7	1537.5 * ±149.0

Values are mean +/- S.E.M.
* indicates values significantly different from appropriate control values at $p < 0.05$

TABLE X

BASAL EFFECTS OF MUSCARINIC BLOCKADE : PACING STUDY

OXYGEN CONTENT AND DERIVED VALUES

	Oxygen Content ml O2/100 ml blood			% O2 Extraction	Left Ventricular $mlO_2/min/100g$	
	Arterial	Venous	A-V		Consumption	Delivery
CONTROL n=10	14.3 ±0.6	3.7 ±0.3	10.6 ±0.5	73.84% ±2.22%	13.93 ±1.36	19.33 ±2.30
PROPRANOLOL PHENTOLAMINE n=10	13.7 ±0.7	3.1 ±0.3	10.6 ±0.6	77.26% ±2.07%	12.83 ±0.90	16.87 ±1.60
ATROPINE n=10	13.8 ±0.7	3.5 ±0.4	10.3 ±0.9	73.90% ±3.71%	11.69 ±0.84	16.22 ±1.50

Values are mean ± S.E.M.

TABLE XI

BASAL EFFECTS OF MUSCARINIC BLOCKADE

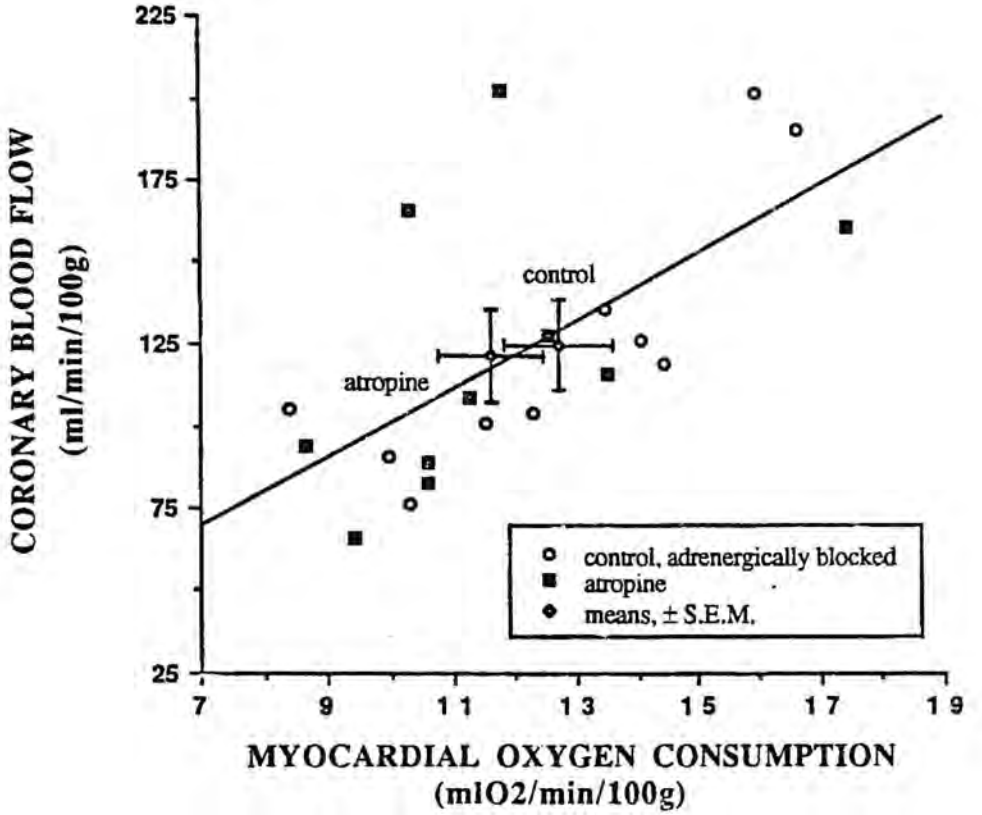
BLOOD GASES

PACED STUDIES

	pO2		pCO2		pH		HEMATOCRIT	
	A	V	A	V	A	V	A	V
CONTROL	83.32 ±3.60	23.61 ±1.58	36.72 ±2.62	46.10 ±2.55	7.46 ±0.02	7.40 ±0.01	32.8 ±5.1	33.7 ±3.2
PROPRANOLOL PHENTOLAMINE	77.67 ±3.53	22.03 ±2.14	33.21 ±2.16	43.15 ±2.00	7.48 ±0.02	7.42 ±0.01	33.3 ±3.9	33.2 ±3.3
ATROPINE	75.86 ±4.81	22.75 ±2.07	33.29 ±1.84	41.90 ±2.01	7.49 ±0.01	7.44 ±0.01	32.6 ±4.5	31.4 ±3.7

Values are means ± S.E.M.

Figure 12. Coronary blood flow and vascular resistance data from open-chest, paced animals are plotted with respect to myocardial oxygen consumption. Individual points are plotted for control (■) and following intracoronary injection of 200 μg atropine (○). Means ± S.E.M. before and after atropine are also presented. Linear regression performed for each group yield regression lines which were not statistically different. Therefore, the calculated linear regression line presented represents the combined data for both groups. The relationship between coronary blood flow and myocardial oxygen consumption ($r=0.66$, $m=10.45$, y-intercept=-3.56) was significant ($p< 0.05$) as was the relationship between vascular resistance and myocardial oxygen consumption ($r=0.46$, $m=0.04$, y-intercept=1.14, $p<0.05$).


CORONARY BLOOD FLOW
(ml/min/100g)
225
175
125
75
25
control
atropine
control, adrenergically blocked
atropine
means, ± S.E.M.
7
9
11
13
15
17
19
MYOCARDIAL OXYGEN CONSUMPTION
(mlO2/min/100g)

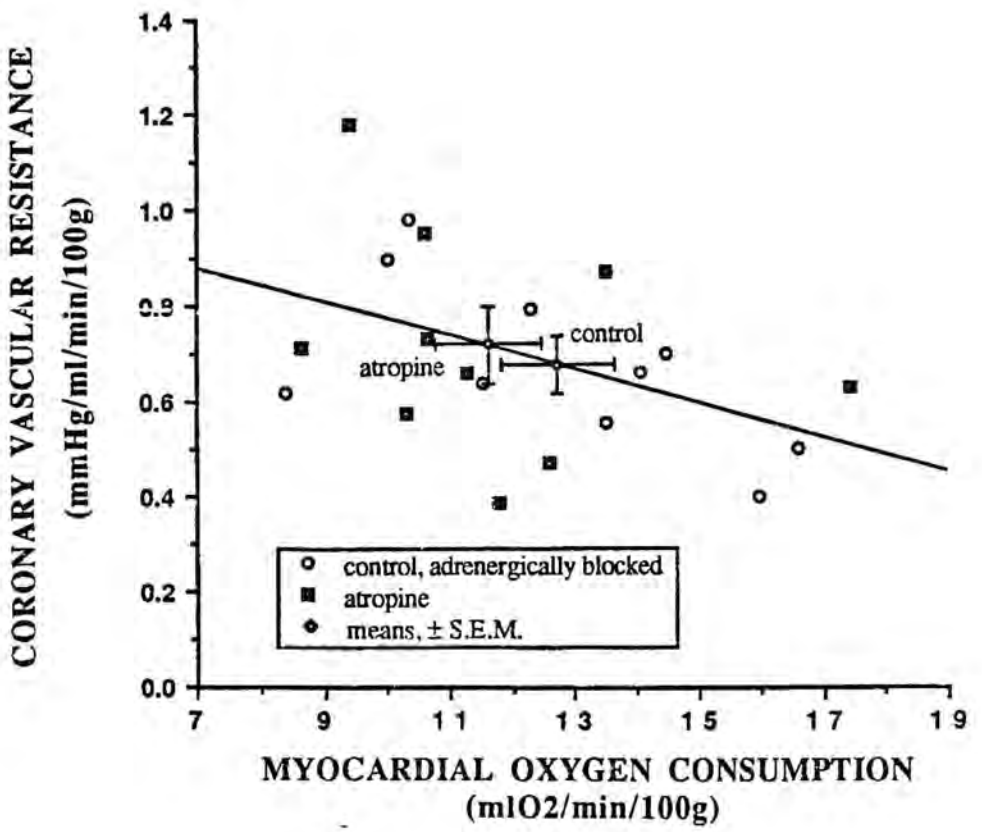
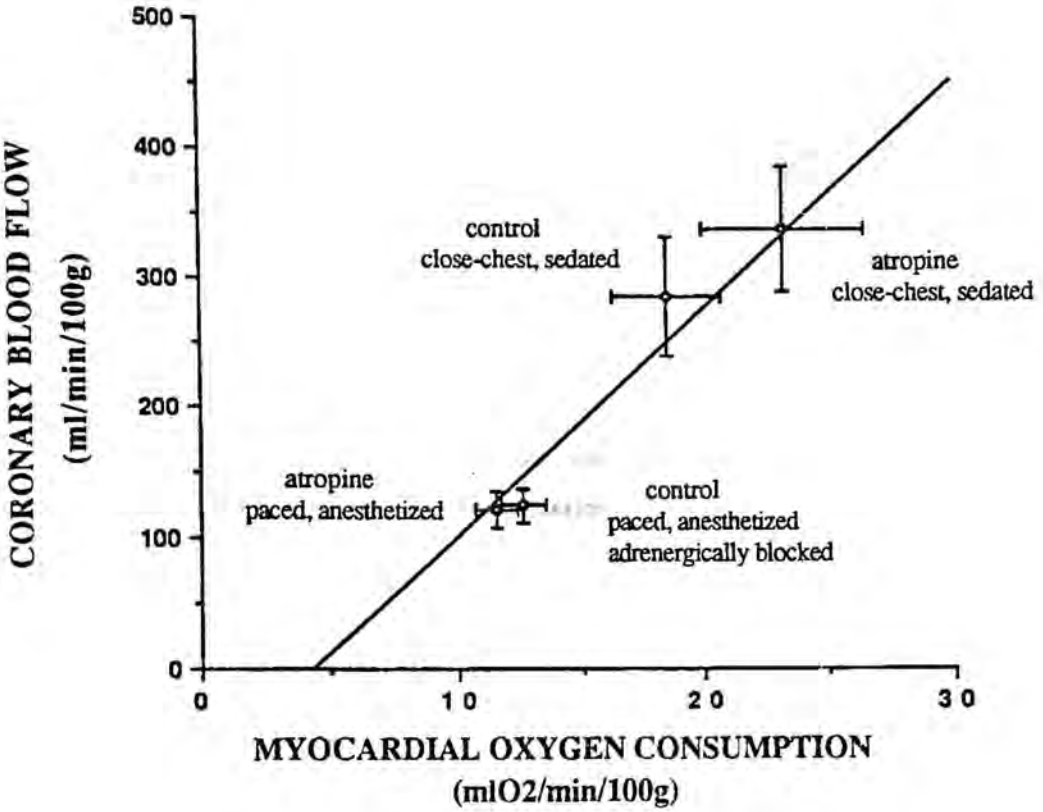

CORONARY VASCULAR RESISTANCE
(mmHg/ml/min/100g)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
control
atropine
control, adrenergically blocked
atropine
means, ± S.E.M.
7
9
11
13
15
17
19
MYOCARDIAL OXYGEN CONSUMPTION
(mlO2/min/100g)

Figure 13. Coronary blood flow and vascular resistance are again plotted with respect to myocardial oxygen consumption. Values are means ± S.E.M. for control and following atropine. Regression line was calculated from the combined control data of closed-chest, sedated and open chest, paced. The relationship between coronary blood flow and myocardial oxygen consumption ($r=0.78$, $m=17.7$, y-intercept=-77.3)($n=16$) was significant ($p< 0.05$) as was the relationship between vascular resistance and myocardial oxygen consumption ($r=0.69$, $m=0.033$, y-intercept=1.07, $p<0.05$)($n=16$).


CORONARY BLOOD FLOW
(ml/min/100g)
500
400
300
200
100
0
0
10
20
30
control
close-chest, sedated
atropine
close-chest, sedated
atropine
paced, anesthetized
control
paced, anesthetized
adrenergically blocked
MYOCARDIAL OXYGEN CONSUMPTION
(mlO2/min/100g)

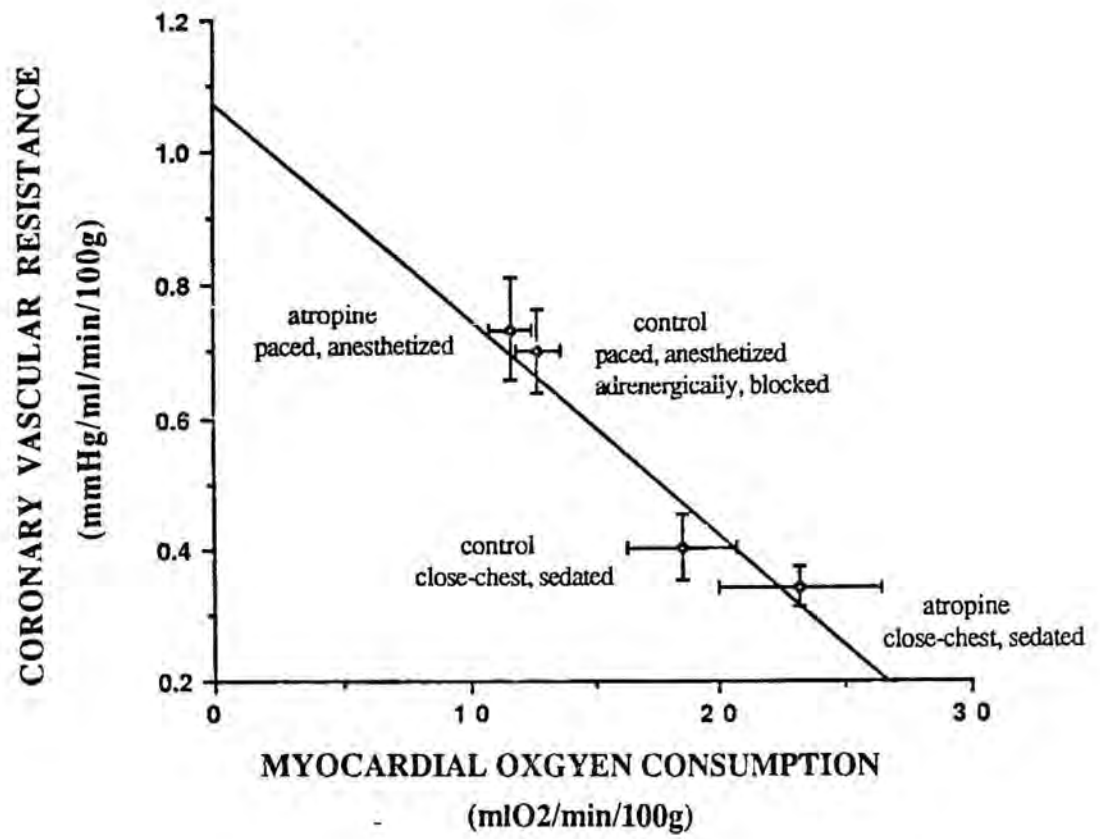

CORONARY VASCULAR RESISTANCE
(mmHg/ml/min/100g)
1.2
1.0
0.8
0.6
0.4
0.2
0
10
20
30
atropine
paced, anesthetized
control
paced, anesthetized
adrenergically, blocked
control
close-chest, sedated
atropine
close-chest, sedated
MYOCARDIAL OXGYEN CONSUMPTION
(mlO2/min/100g)

TABLE XII

CORONARY BLOOD FLOW AND RESISTANCE

VAGAL LIGATION, PACED

SAMPLE	Left Anterior Descending Coronary Flow ml/min/100g	Resistance mmHg/ml/min/100g
CONTROL n=6	175.1 ±22.5	0.549 ±0.091
RIGHT VAGAL LIGATION n=6	175.5 ±22.1	0.517 ±0.078
CONTROL 2 n=6	164.7 ±20.3	0.522 ±0.073
LEFT VAGAL LIGATION n=6	172.6 ±18.3	0.462 ±0.071

Values are mean ± S.E.M.
* Indicates significantly different from the appropriate control values at $p < 0.05$
** indicates significant difference between complete vagal ligation and initial control at $p < 0.05$

TABLE XIII

VAGAL LIGATION, PACED

HEMODYNAMICS

SAMPLE	Heart Rate beats/min	Blood Pressure mmHg	Peak Systolic Pressure mmHg	Pressure Rate Product mmHg/min	dP/dt mmHg/min
CONTROL n=6	158.8 ±4.4	84.9 ±6.1	100.5 ±6.0	16101.8 ±1346.5	1835.5 ±115.2
RIGHT VAGAL LIGATION n=6	161.0 ±5.8	82.1 ±3.9	96.8 ±4.2	15864.2 ±1186.0	1895.8 ±119.4
CONTROL 2 n=6	161.2 ±5.8	77.9 ±4.1	92.5 ±4.1	14961.7 ±997.4	1845.8 ±81.3
LEFT VAGAL LIGATION n=6	161.2 ±6.5	72.9 ±5.2	89.0 ±4.4	14452.2 ±1192.4	1979.2 ±145.6

Values are mean ± S.E.M.
* indicates significant difference
from appropriate control values at $p < 0.05$
** indicates significant difference
between complete vagal ligation and initial control at $p < 0.05$

TABLE XIV

VAGAL LIGATION, PACED STUDIES

OXYGEN CONTENT

	Oxygen Content				Left Ventricular	
	Arterial	Venous	A-V	%O2 extraction	Consumption	Delivery
	$ml O_2$/100ml blood				$ml O_2$/min/100g	
CONTROL	13.0	2.1	10.9	84.04%	19.1	22.7
n=6	±1.5	±0.4	±1.2	±2.05%	±2.7	±1.9
BILATERAL						
LIGATION	13.2	2.3	10.9	82.96%	18.8	22.8
n=6	±1.3	±0.4	±1.0	±1.84%	±2.2	±1.8

Values are mean ± S.E.M.
* indicates values significantly different from control at $p < 0.05$

TABLE XV

BLOOD GASES:

VAGAL LIGATION STUDIES

	pO2		pCO2		pH		HEMATOCRIT	
	A	V	A	V	A	V	A	V
CONTROL	93.2 ±10.3	17.7 ±1.8	37.3 ±3.8	44.4 ±3.6	7.48 ±0.04	7.43 ±0.03	29.6 ±1.9	30.1 ±2.2
BILATERAL VAGAL LIGATION	96.6 ±6.2	20.1 ±1.4	37.3 ±7.5	45.8 ±3.0	7.46 ±0.02	7.42 ±0.02	32.5 ±1.8	31.3 ±1.8

Values are means ± S.E.M.

DISCUSSION

These studies were designed to determine the role of the tonic parasympathetic activity to the heart in mediating a basal coronary vasoconstriction, or tone, capable of limiting oxygen delivery to the myocardium. Whether the basal cholinergic influence is distributed uniformly across the myocardium was also tested. Preliminary studies determined that acetylcholine is a potent vasoconstrictor of porcine coronary arteries *in vivo*. However, these studies did not provide evidence for a role of tonic parasympathetic activity in mediating basal coronary tone.

Previous studies of neural involvement in coronary tone have focused on adrenergic mechanisms because alpha adrenergic vasoconstriction has been well documented. The recent appreciation of acetylcholine as a vasoconstrictor, in species other than the dog, suggests that tonic parasympathetic activity could contribute to resting coronary tone. Although there is much evidence from *in vitro* studies of isolated vessels to indicate that acetylcholine is a potent vasoconstrictor [30,32,33,50,52,74,75] the effect of acetylcholine on the intact porcine coronary circulation had not been determined.

Intracoronary injection of acetylcholine in the present *in vivo* studies on domestic swine produced dramatic reductions in coronary flow and increases in resistance (Figure 6). Acetylcholine in doses ranging from 0.5 to 3.0 μg induced significant reductions in coronary flow. The maximum reduction in mean coronary blood flow was 75% produced by 2.5 μg

acetylcholine. These findings are in close agreement with the findings of Young et al. [108] who found 71% reduction in coronary blood flow of calves with a similar dose of acetylcholine.

Unlike Young's study in which the reduction in flow had no significant effects on mean arterial pressure, in the present studies acetylcholine produced significant reductions in mean arterial and peak systolic pressure and dP/dt. Although the hemodynamic changes were statistically significant, they were relatively modest compared with the dramatic changes in coronary flow. Mean coronary blood flow following a 2.0 μg dose of acetylcholine, as depicted in Figure 7, was reduced 70% while intraventricular and mean arterial pressure were only reduced 5.1% and 7.7%, respectively. Also, lower doses of acetylcholine (0.5μg) produced significant changes in flow than was required to induce changes in mean arterial or peak systolic pressure (1.0μg). It is unlikely therefore that the modest reduction in perfusion pressure or myocardial metabolism that accompanied the hemodynamic changes played a role in the dramatic decrease in coronary flow following acetylcholine. Conversely, it is possible that the severe reduction in coronary flow had a negative effect on myocardial performance. Vatner [99] and Downey [15] have shown that reductions in coronary flow below the level determined by autoregulation can cause a significant reduction in contractile force. Acetylcholine's potent vasoconstrictor nature in isolated vessels, as well as in calves, suggests that the dramatic flow reduction in these studies following acetylcholine is not a secondary response caused by reduced perfusion pressure or myocardial metabolic demand but rather a direct vasoconstriction.

Damage to the vascular endothelium has been shown to reverse the vasodilation that is normally produced by acetylcholine, to vasoconstriction [26]. Therefore, possible damage of the endothelium by introduction of the modified Herd-Barger catheter into the left anterior descending coronary artery in the acetylcholine dose-response studies could have resulted in acetylcholine's vasoconstrictor effects. However, this would seem unlikely in the face of multiple demonstrations of vasoconstriction to acetylcholine with intact endothelium in human, porcine, and cattle isolated vessels [32,33,52,53,74,75,93].

Another possible mechanism for muscarinic reductions in coronary blood flow has been suggested to be through presynaptic inhibition of norepinephrine release [11]. Acetylcholine has been shown to reduce the amount of norepinephrine released upon transmural stimulation of isolated vessels [10]. The reduction in norepinephrine release would result in vasoconstriction if the predominant action of the catecholamine is beta adrenergic vasodilation. Although *in vitro* studies have shown norepinephrine, either exogenous or neurally released by transmural electrical stimulation, to cause beta adrenergic relaxation [10,50,75] the effects of norepinephrine release *in vivo* remains unclear. Several studies indicate that adrenergic receptor subtypes vary along the coronary arteries with predominantly alpha adrenoceptors in the large epicardial arteries, while beta adrenoceptors occupy small resistance vessels [4,50,72,70,75]. However, *in vivo* studies, without prior beta-adrenergic blockade, display alpha-adrenoceptor mediated reductions in coronary flow following catecholamine injection or stellate ganglion stimulation [39,100]. Although the effects of adrenergic blockade were not

investigated in these studies, it would seem unlikely that modulation of adrenergic influences is the mechanism by which acetylcholine induces such potent vasoconstriction. Several *in vitro* and *in vivo* studies have provided evidence that prior administration of adrenergic antagonists has no effect on the vasoconstriction produced by acetylcholine [27,75,86,108].

Response in all parameters to intracoronary injection of acetylcholine (0.5 to 3.0 μg) in this study was blocked by atropine (Figures 7,8). The vasoconstriction produced by high doses of acetylcholine was greatly attenuated. The competitive nature of this blockade has been demonstrated by Ito et al.[50] in porcine coronary vascular smooth muscle and by Nakayama et al.[75] in isolated porcine coronary resistance vessels. Results of these and other studies [27,54,108] indicate that acetylcholine released from parasympathetic nerve terminals on the coronary arteries mediates vasoconstriction through muscarinic receptors on the vascular smooth muscle and that this response is not dependent upon an alteration of adrenergic responses.

Existence of a tonic cholinergic vasoconstriction mediated via muscarinic receptors was investigated by intracoronary injection of 200 μg atropine. This dose was determined to provide effective blockade against high doses of intracoronary acetylcholine, while intracoronary administration avoided systemic effects. When the dosage is standardized by division of body weight, this dose is approximately 50-times less than that given systemically in other studies [51,69,108]. However, since the drug was given intracoronary, division of the dose by heart weight yields a 4-fold greater dose delivered to the cardiac tissue. Thus, 200 μg atropine intracoronary provides effective blockade

of the actions of acetylcholine in high doses, without having significant systemic effects.

Initially, a sedated closed-chest pig model was chosen to avoid the adverse effects of anesthesia and the sympathetic stimulation associated with surgical trauma [98]. Diazepam sedation was chosen for it's relative lack of cardiovascular [2,34] effects and widespread clinical use in similar catheterization procedures [2,40].

In this model a significant increase in basal coronary blood flow and reduction in vascular resistance was observed following atropine (Table IV). According to the working hypothesis, muscarinic blockade of a cholinergic tone which limits coronary flow would be expected to increase both coronary sinus oxygen content and blood flow at any given rate of myocardial oxygen consumption. However, this was not the case. Intracoronary injection of atropine also resulted in an increased heart rate and pressure rate product which are likely to have resulted in the significant increase in myocardial oxygen consumption. Heart rate and pressure rate product changes will be discussed more thoroughly later. The increased myocardial oxygen consumption caused an increased oxygen extraction which left the coronary sinus oxygen content unchanged in the face of increased delivery. The relationship of coronary blood flow and resistance with myocardial oxygen consumption also was not changed by removal of muscarinic influences (Figure 11).

The slope of the regression lines for these curves, before and after atropine, were not significantly altered. The increased flow following atropine was matched by increased oxygen consumption. This relationship is illustrated by the rightward movement of the means along

the regression line. Increased myocardial oxygen consumption has been shown repeatedly to increase coronary blood flow through metabolic vasodilation [3,65,79]. Thus, it appears that the change in coronary flow following atropine was a result of increased metabolic demand rather than removal of a direct parasympathetic vasoconstrictor effect.

Epicardial and endocardial flows also increased significantly, however, the increase in epicardial flow was greater than that of the endocardium resulting in a significant reduction in the endocardial/epicardial flow ratio. This relationship was mirrored by vascular resistance in which epicardial resistance decreased significantly while the decrease in endocardial resistance was not significant. Reduction in the endocardial/epicardial flow ratio is the normal response to increased in myocardial oxygen demand [48]. Thus, transmural flow changes lend support to the interpretation that an increased myocardial oxygen demand caused the increase in coronary blood flow.

Coronary flows in this study are approximately twice those reported by others for conscious swine whereas the present heart rates and blood pressures are quite similar [19,71]. Erroneously high coronary blood flows as measured by microspheres could result from; 1.) a low non-representative arterial withdrawal sample, 2.) insufficient microspheres per tissue sample, 3.) streaming of the microspheres into the coronary circulation, 4.) undervalued weight measurements. The arterial withdrawal sample was taken from a catheter placed in the axillary artery via the mammary artery. Position of the catheter was verified by injection of radio-opaque dye at the time of coronary artery

catheterization. Blood was withdrawn at a rate of 2.09 ml/min which resulted in over 400 microspheres in the sample. Also, microspheres were injected in concentrations which were sufficient to yield 400 microspheres per tissue sample, a concentration described to allow measurement of flow to within 10%. This has been shown to enable flow determinations within 10% of the mean distribution with 95% confidence [7]. Position of the coronary artery catheters was determined not to have an effect on the coronary flow as determined by microsphere injections before and after catheter placement in two animals. Also, percent cardiac output to the heart compared favorably with previously published data [94], further indicating that streaming of microspheres into the coronaries did not result in an elevated measurement of percent of cardiac output to the heart and therefore flow. Lastly, absorption and evaporation of fluid from the myocardial tissue samples as they were being weighed could conceivably have resulted in a very slight underestimation of tissue weight, however it seems unlikely that this could account for a two-fold overestimation of flow. Although steps were taken to insure accurate microsphere determination of blood flow, methodological factors cannot be ruled out. Rosenberg et al.[83] found microsphere flow measurements to be overestimated when small lumen catheters in peripheral arteries were used to collect the arterial reference sample from animals with hematocrits less than 32% at a withdrawal rate below 2.46 ml/min. This could be a factor in the present studies in which arterial reference samples were withdrawn through tygon tubing (0.04 in. I.D., 0.07 in. O.D.) at a rate of 2.09 ml/min and the hematocrits averaged 21.9 ± 4.4. Although absolute

values were higher than previously reported values, the changes in flow correlated well with changes in heart rate (r=0.81), pressure rate product (r=0.92) and oxygen consumption (r=0.57), indicating that flow measurements were at the very least qualitatively accurate.

It was felt that had a tonic cholinergic vasoconstriction been removed during the closed-chest studies, the resultant modest increase in flow may have been obscured by the unexpected increase in myocardial oxygen consumption which increased flow through indirect mechanisms. Therefore, a second model was developed to test the effects of muscarinic blockade under conditions of constant myocardial demand. An open-chest anesthetized model was used which allowed for control of heart rate through sequential atrial-ventricular pacing. The effect of possible increased sympathetic activity caused by surgical trauma on the vascular adrenergic receptors, was blocked by administration of alpha and beta antagonists, phentolamine and propranolol, respectively. The administration of these adrenergic blocking agents was expected to alter the vascular response to acetylcholine, since several studies have shown this response to be independent of adrenergic receptors [50,54,89,108]. Since an open chest model was used it was necessary to anesthetize the pigs. The combination of alpha-chloralose and urethane was chosen for it's lack of inhibition of autonomic reflex responses [34,95].

When changes in myocardial performance were maintained constant by pacing and adrenergic blockade, atropine had no effect on basal coronary flow or resistance. There also was no significant increase in coronary sinus oxygen content. A significant relationship existed, as it did in

the previous study between coronary flow and resistance and myocardial oxygen consumption (Figure 11). The slopes of the regression lines before and after atropine were not significantly different, indicating that atropine did not alter the relationship between consumption with flow and resistance. The absence of an increased coronary flow or alternatively an increased coronary sinus oxygen content at any given oxygen consumption following atropine indicates that there is no tonic cholinergic vasoconstriction which limits resting coronary blood flow in this model.

Significant changes did occur in the endo/epi-cardial blood flow ratio and the rate of change of left ventricular pressure, dP/dt. Although neither regional flow changed significantly, each was reduced slightly with endocardial flow being reduced more than epicardial, resulting in a slight (4%) yet significant reduction in the transmural flow ratio. Although, a significant difference was found in the transmural flow, the small magnitude of the change clouds the possible physiological importance of this finding.

The mean values from both sedated, closed-chest and anesthetized, open-chest studies, before and after atropine, for myocardial blood flow and resistance and oxygen consumption, fell along a linear regression line of control values from both groups (Figure 13). This suggests that these two studies had a comparable relationship between these parameters. Atropine caused a significant rightward shift along this regression line in the sedated study, indicating that changes in flow were matched by changes in myocardial oxygen consumption. Conversely, there was no significant movement along the regression line in the

anesthetized paced study. This comparison lends further support to the conclusion that removal of cholinergic influences had no direct effect on coronary vascular tone.

Significant increases in parameters of myocardial function occurred following atropine in both the sedated closed-chest and anesthetized open-chest studies. Spontaneous heart rate was increased in the former, which led to a significant rise in pressure rate product. In the latter study, in which heart was controlled and adrenergic receptors blocked, dP/dt increased significantly following atropine. In the first study heart rate could have risen due to removal of muscarinic inhibitory influences on the sino-atrial node. It would seem improbable that atropine administered into the left coronary artery in relatively trivial systemic amounts could have recirculated to act on the sino-atrial node. Although also unlikely, the possibility that atropine refluxed from the left coronary artery into the right coronary which supplies the nodal tissue can not be ruled out. In the latter study, it would be doubtful that the increase in dP/dt which occurred following atropine could have been due to the removal of a presynaptic inhibition of norepinephrine release from adrenergic nerve terminals [10], because adrenergic blockers had previously been administered. An alternative explanation is that acetylcholine was acting on the myocardium to directly reduce it's performance. Indeed, stimulation of the vagus or injection of acetylcholine has been shown to produce direct modest reductions in conduction velocity and myocardial performance [13,59]. Removal of such a tonic cholinergic inhibitory influence would therefore result in the observed increased performance.

Although atropine readily blocks intracoronary effects of acetylcholine it is less effective in counteracting the actions of endogenous acetylcholine acting on muscarinic receptors in the perivascular region of the heart. This conclusion is supported by the findings of Young et al. [108] and Furusho et al. [27], which demonstrate a complete abolition by atropine of coronary flow reductions to intracoronary acetylcholine, yet only a 10% reduction in the effects of cholinergic neural stimulation. Thus, it appears that the physiologic importance of acetylcholine in regulating coronary vascular tone can not be fully revealed through the use of atropine alone. For this reason, an alternative method for removing cholinergic activity to the heart, vagal ligation, was carried out to test for the presence of parasympathetic influences that are resistant to atropine.

Vagal ligation was carried out in nine open-chest alpha-chloralose/urethane anesthetized swine. As an indicator of parasympathetic activity to the heart, the vagi of three animals were ligated and changes in spontaneous heart rate were observed. Following ligation of both right and left vagal nerves, heart rate tended to increase approximately 23 beats per minute. This observation supports the presence of tonic parasympathetic activity to the heart. In the six remaining animals, heart rate was maintained constant with sequential pacing. Section of the right and left vagal nerves had no effect on coronary blood flow, vascular resistance or any hemodynamic parameter. Once again, had tonic coronary vasoconstriction been vagally mediated, an increase in coronary flow would have been expected following vagal ligation. Also, there was no change in the oxygen extraction or

myocardial oxygen consumption (Table XIV). These observations lend further support the conclusion that tonic parasympathetic activity to the heart does not mediate a tonic vasoconstriction on resting coronary blood flow.

That parasympathetic ligation had no effect on any hemodynamic parameter would suggest that the tonic cholinergic effects detected in the earlier studies were not due to parasympathetic activity. This could indicate that the cholinergic inhibitory effects removed by atropine were mediated by a basal level of acetylcholine present due to spontaneous release of individual acetylcholine storage vesicles from cholinergic nerve endings [61]. Loffelholz [60] has measured basal levels of acetylcholine released from isolated hearts. Acetylcholine can be measured in the perfusate of the isolated heart partly due to it's relatively slow rate of hydrolysis in the heart [60]. Acetylcholine released in a similar fashion could account for Kalsner's [52] finding that although atropine had no effect on basal coronary tone, whereas the anticholinesterase, physostigmine, increased vascular tone in a denervated slab of beef heart.

The present *in vivo* studies provide support from the porcine coronary circulation for the vasoconstriction to exogenous acetylcholine observed in isolated porcine coronary arteries [27,33,50,74,75]. The increased tension development of isolated vessels [74] is sufficient to alter flow in the intact coronary circulation. Cholinergic vasoconstriction as demonstrated in these studies is in agreement with the findings of Young et al.[108] in calves. Acetylcholine has also been

shown to be a potent vasoconstrictor in humans [47,68,77,78,104]. Although much of this work has focused on vasospasm of diseased coronary arteries, intracoronary injection of acetylcholine into angiographically normal coronary arteries also produces substantial vasoconstriction [68,77,104]. The cholinergic vasoconstrictor results of the present studies may be added to the growing evidence in support of acetylcholine's ability to reduce coronary flow and in contrast to the previously accepted vasodilator capacity which had been determined in the dog.

A limitation of the acetylcholine dose-response study was the inability to separate the reduction in flow from decreases in pressure. Although acetylcholine has negative inotropic effects, it is unlikely that those in this study were of sufficient magnitude to account for the flow reductions. On the other hand, Vatner [99] found significant reduction if function with 10-20% decreases in flow. The significant flow changes induced by acetylcholine in these studies were 20% (0.5 μg) or greater, suggesting ample reduction in flow to account for the decreased function. However, no conclusion concerning cause and effect can be made from these studies.

The cholinergic tone studies were limited by the necessity to use either sedation or anesthesia, both of which may reduce the parasympathetic tone to the heart [28,45,56]. Therefore, although tonic parasympathetic activity to the heart was evidenced in both studies of basal cholinergic tone employing muscarinic blockade, greater parasympathetic activity to the heart may exist in the conscious resting animal. Also, these studies investigated the effects of muscarinic blockade or vagal ligation on global coronary flow. It may be that

cholinergic vasoconstrictor influences are greater in some regions of the heart than in others [14,66]. Particularly, cholinergic innervation of the left circumflex coronary artery appears to be greater than the left anterior descending coronary artery.

Difficulty in determining the regulation of basal coronary blood flow is evidenced by the number of studies which have been conducted to investigate the mediators known to regulate coronary flow under stimulated conditions. This research has failed to identify a definitive role for any agent in the regulation of basal coronary flow. A good example of this is adenosine, a potent metabolic vasodilator which has been shown to mediate increased coronary flow during states of high myocardial metabolic activity [3,65,79,85], yet, no role has been attributed to it in the regulation of basal coronary flow [29,57]. Adrenergic influences have been shown to limit coronary flow during states of high sympathetic activity [37,38,48,51,69,73]. Nevertheless, attempts to demonstrate adrenergic participation in basal coronary tone have resulted in conflicting findings [9,36,46,63].

It is likely that determining a factor as the sole regulator of basal coronary blood flow is difficult because the level of basal coronary blood flow is the result of multiple regulatory factors. That vascular smooth muscle exists in a state of tonic contraction is well accepted [6]. However, no putative mediator has been demonstrated to be responsible for this tonic vasoconstriction. It may be that basal coronary tone is an intrinsic characteristic of the vascular smooth muscle. The dependence of the contraction on intracellular calcium concentrations has also been well established [50]. The level of

intracellular calcium and therefore the tone of the vascular smooth muscle is altered by many factors. The following factors, as well as others, act individually or in combination to modulate basal coronary tone; arterial pressure, endothelium-dependent relaxing factor, adenosine, calcium ions, arterial oxygen content, pH, acetylcholine, norepinephrine, prostaglandins, or histamine. The integration of all mechanical and chemical signals at any given instant, therefore, would result in an appropriate basal coronary tone.

The similarities that exist between the coronary arteries of swine and man suggests that the present findings may also extend to human coronary physiology. Forstermann [23] demonstrated vasoconstriction of isolated human coronary arteries free of atherosclerosis. However, studies demonstrating cholinergic coronary vasoconstriction in humans are primarily in patients suffering from angina. The results of the present findings suggest that acetylcholine may cause coronary vasoconstriction in normal humans as well. The lack of basal cholinergic influence on resting coronary tone suggests that the coronary vasospasm associated with high parasympathetic activity in Prinzmetal's angina may be due to some pathological removal of an compensatory vasodilator. The angina might also result from an increased sensitivity to, or activity of, the parasympathetic innervation.

The results of the studies presented extend the basic understanding of cholinergic regulation of coronary blood flow. Acetylcholine produced significant reductions in coronary blood flow indicating coronary vasoconstriction. Removal of cholinergic influence through muscarinic blockade or vagal ligation had no effect on resting

coronary blood flow suggesting that basal coronary tone is not regulated by cholinergic mechanisms.

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